Biology of the Prostate and Related Tissues

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WORKSHOP ON
THE BIOLOGY OF THE PROSTATE
AND RELATED TISSUES

Sponsored by
Cancer Chemotherapy National Service Center
National Cancer Institute
National Institutes of Health
Bethesda, Maryland

Editor: Erwin P. Vollmer
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Acknowledgments

This program was supported by a research grant (CYP-5625) from the National Cancer Institute to Dr. Elwood V. Jensen, chairman of the workshop. At the time the grant was made, Dr. Jensen was chairman of the Endocrinology Panel, one of several panels advisory to the Cancer Chemotherapy National Service Center prior to 1962, which had recommended a program of this type as a source of new approaches to the problems of prostatic cancer. Dr. Stuart M. Sessoms' support in furthering the project was essential and greatly appreciated.

On behalf of the institutions served, the editors wish to thank the participants, whose work makes up the substance of the monograph, and the committee whose planning is reflected in the contents. They also wish to thank the editorial staff of the Journal of the National Cancer Institute. Finally, they wish to thank Georgia F. Becker and Mary L. Jiuliano for typing the discussions from sound transcriptions; Arthur Hilgar, Donald Hummel, Ihor Masnyk, and D. Jane Taylor for reading manuscripts; and Frank Dondero, Margaret Lamphier, Chester Piczak, and Barbara Stevenson for their assistance, with the other members of the Endocrine Evaluation Branch of CCNSC, with a great variety of other tasks that had to be carried out.

The workshop was held at Airlie House in Warrenton, Virginia, from October 1-3, 1962.

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INTRODUCTION[1]

CHARLES HUGGINS, The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois

In an old fable a group of blind men encountered an elephant for the first time, and each was impressed with a different characteristic of the great beast. So it is when one approaches a great area of science in which knowledge is fragmentary and primitive. Such an area is the biology of prostatic cells. In science the journeyman specialist studying a problem in an elementary state of knowledge is apt to obtain a restricted view of what he finds, based largely on and conditioned by his early training and his personality. One purpose of a symposium of this sort is to assemble the bits and pieces of prostatic problems in the hope that a more unitary concept may emerge.

Why has this group of eminent specialists convened? Let us be blunt. Whereas it is pleasant for a group of amiable and knowledgeable people to gather in a beautiful place, sheer pleasure is not the overriding consideration for this meeting. The clue comes from the sponsors. This Workshop is sponsored by the Cancer Chemotherapy National Service Center in the unexpressed, but frank, hope that from the discussions some new knowledge will emerge that will be of value to man sick with prostatic cancer. It is a serious assignment. One could wish for the presentation of a diagnostic or therapeutic discovery or for fundamental building stones which would pave the way for such discoveries. It must be remembered that scientists are the sole repository of the hopes and prayers of millions of people for advances in understanding and cure of cancer and allied diseases.

Perhaps it is appropriate to set the problem of the human prostatic tumors in proper perspective. In the Western world prostatic tumors are very common. In the United States more than one half of the male population over 50 suffer (the verb is used advisedly) from benign tumors known as prostatic hypertrophy. Often an enlarged prostate is the only difficulty to cloud an otherwise tranquil old age. Likewise, prostatic cancer is the commonest cancer of men older than 50.

The biology of the prostatic glands is full of fascination—the prostate does nothing by halves. An eminent student of the biochemistry of the reproductive tract, Dr. H. G. Williams-Ashman, has stated (*1*): "There are two attributes of the male accessory glands which make them of extraordinary interest to the biochemist. First, their normal development and function are utterly dependent upon steroid hormones. Second, the chemistry of their secretions is often rather strange, and sometimes very odd indeed."

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

Several unsolved problems of neoplasms of the prostate can be pointed out.

1) As far as is known, prostatic tumors, benign or malignant, occur spontaneously only in man and dog.

2) Prostatic cancer constitutes an interesting problem in geographic pathology. In the United States in 1959, 126,478 white males were reported (*2*) to have died from cancer and of these, 12,280 deaths (9.7%) were from prostatic cancer; in the nonwhite population the mortality was similar and 13.9 percent of the cancer deaths were from cancer of the prostate. But in Japan, in 1959, the death rate of prostatic cancer (0.9%) was remarkably lower than in the United States.

3) Whereas cancer of the prostate is common in man, carcinoma of the seminal vesicle is a medical rarity. Both structures are dependent on testosterone and both are adjacent in site.

4) Commonly, cancer of the human prostate occurs only in the periphery of this organ, while the center of the prostate is largely or entirely devoid of the capacity to undergo malignant change.

5) The incidence of prostatic cancer in aged white males approaches 100 percent of the population.

The agony of the advancement of medicine has been expressed by F. Sauerbruch who said in effect: "Those who know cannot cure, and those who cure do not know." The chief purpose of symposia of this kind is to try to overcome this vexatious dilemma and to bring advances in science to the bedside.

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COMPARATIVE MORPHOLOGY

Chairman: James H. Leathem

COMPARATIVE ASPECTS OF DEVELOPMENT AND STRUCTURE IN THE PROSTATE[1, 2]

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The accessory reproductive glands have been organs of especial interest to nonmedical and medical scientists for many years. Much of the experimental work has been stimulated by the problem of prostatic carcinoma and benign hyperplasia in man. A large literature has accumulated and recently has been partly reviewed (*1*, *2*). However, there is still some confusion in the nomenclature of the prostatic lobes or regions and in their homologies. This paper deals specifically with this problem and with certain aspects of the responsiveness of the lobes to hormones.

BRIEF SURVEY OF ACCESSORY REPRODUCTIVE GLANDS

All male mammals possess one or more accessory reproductive glands, which have been named for their anatomical position in adult animals or for their assumed or demonstrated special functions. Examples of the first category are such glands as the prostate, paraprostate, ampullary, bulbourethral (Cowper's), and bulbar; of the second category, seminal vesicles (a misnomer) and coagulating glands. The only gland found in all orders of mammals, even in monotremes, is the prostate (*2*). There are marked differences between orders in respect to the accessory glands present. For example, carnivores have no seminal vesicles, sirenians (dugongs and manatees) have neither ampullary nor bulbourethral glands, whereas cetaceans, another aquatic order, lack all three of these glands and have only a prostate. Variability in the glands represented exists even between families within orders.

Homologues of two of the male accessory glands, the prostate and bulbourethral, are found in adult females in several orders and are the female prostate (glands of Skene) and bulbovestibular (Bartholin's) glands, respectively. The occurrence of female prostates has been discussed recently (*2*).

Text-figure 1 illustrates the gross anatomy of the male accessory glands in representatives of four orders of mammals. The bull has a

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

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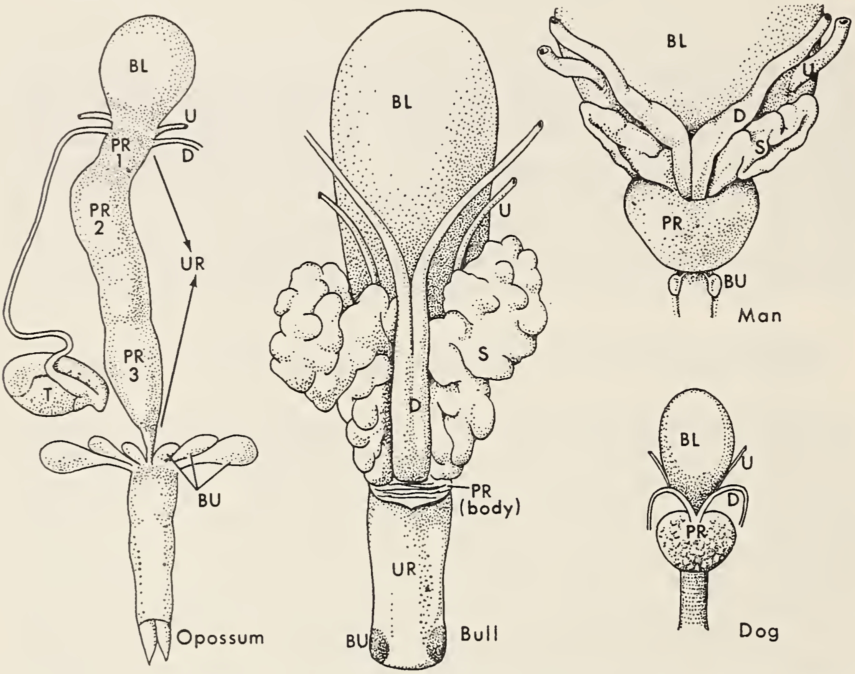
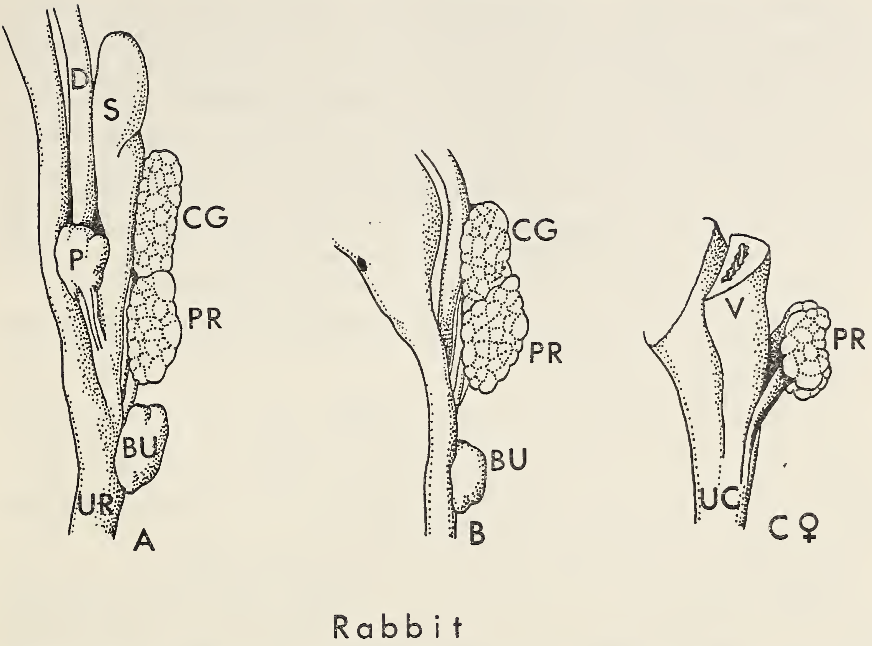
full quota, with seminal vesicles, ampullary enlargements at the base of each ductus deferens, prostate (both an external "body" and a diffuse or disseminate gland surrounding the urethra), and bulbourethral glands. The dog, in contrast, has only a prostate, although the epithelium of the narrow ampullae may be secretory. In the dog and man the prostate is intimately associated with the neck of the bladder, while in the opossum the disseminated prostate gland surrounds the length of the urethra. The reproductive tract of the rat is shown in figure 4 with a complement of accessory glands including seminal vesicles, coagulating glands, and dorsal, lateral, and ventral prostate lobes. The bulbourethral glands are not illustrated.



Text-figure 1.—Accessory reproductive glands in representatives of four orders of mammals. Dorsal view. Key: BL, bladder; BU, bulbourethral glands; D, ductus deferens; PR, prostate; S, seminal vesicle; T, testis; U, ureter; UR, urethra.

Text-figure 2 presents the gross anatomy of the accessory glands in the male domestic rabbit and in the male and female wild cottontail rabbit (*Sylvilagus floridanus*). It depicts the differences in the males of the two species, since the cottontail has no seminal vesicles or paraprostates. On the other hand, the female domestic rabbit has no prostate, while all adult cottontail females examined had large bilobed prostates (*3*).

Some interesting evidence of genetic control of certain glands and also unusual morphologic structure has accumulated. There are marked differences between pure line strains of mice in the size of the ampullary glands, and in strain RIII the glands are only vestigial (*4*). In strain C mice the seminal vesicles and coagulating glands were consistently larger



Text-figure 2.—Lateral view of accessory reproductive glands in rabbits. A, male domestic; B, male cottontail; C, female cottontail. Key: BU, bulbourethral glands; CG, coagulating glands; D, ductus deferens; P, paraprostate; PR, prostate; S, seminal vesicle; UC, urogenital canal; UR, urethra; V, vagina. Bulbovestibular glands are not illustrated in the female. [Text-figure redrawn from Elschlepp (*3*).]

than in mice of other inbred strains and, in addition, the surface of the seminal vesicle was serrated ("prickly") and the coagulating glands were so deeply indented that they appeared ribbed (*5*). A further striking morphologic change was a high incidence of unilateral or bilateral doubling of the seminal vesicles. Since these characteristics did not appear in substrains such as C3H, the possibility was suggested that the characters developed after the substrains were derived from C.

An outstanding example of genetic control of the occurrence of an accessory gland—the prostate— is the demonstration of Witschi and his collaborators (*6*, *7*). They found the incidence of spontaneously occurring female glands in rats could be greatly increased by selective inbreeding.

Congenital anomalies of the accessory glands are seldom found but, if present, are usually associated with other abnormalities of the reproductive tract. Neoplasms too are rare, with the noteworthy exception of those frequently present in the prostate of man and dog. Except in those two species, there are only a few reports of hyperplasia or neoplastic growths. Among tumors found in wild animals kept in captivity, a greatly enlarged prostate was reported in one carnivore (*Paradoxus niger*, a palm-civet) and two prostatic abnormalities were observed in primates—

a papillary adenoma in a ring-tailed lemur (*Lemur catta*) and glandular hyperplasia in a monkey [*Macacus rhesus* (*8*)]. No other accessory glands were affected and there was no cancer. However, a primary carcinoma of the prostate was noted in an old rhesus monkey [*Macaca mulatta* (*9*)].

In stressing the rarity of carcinoma of the prostate in animals, Moore (*10*) reviewed the literature and reported the following incidence: 1 in 110 tumors in bovines; 1 in 509 tumors in horses; no prostatic tumors among 103 primary tumors found in 100,000 wild rats; 1 sarcoma of the seminal vesicle among the many reported tumors of mice, but no prostatic carcinoma; 24 carcinomas in cats, but none in the prostate.

In dogs, there were 11 carcinomas of the prostate among 956 carcinomas (*10*). Zuckerman and McKeown (*11*) studied the prostates in 243 dogs and found only 1 carcinoma.

Cancer of the prostate is common in older men, and by their eighth decade it is one of the most frequent of all carcinomas. Moore (*12*) reported that cancer of the prostate ranked sixth in incidence in a series of 22,139 cancers. It is rare in men under 30. The posterior region of the prostate is the site of origin of carcinoma.

Neoplasms of the seminal vesicle are extremely rare (*14*).

Benign hyperplasia of the prostate, a nodular hyperplasia limited to the inner group of prostatic tubules (fig. 14), occurs in 75 to 80 percent of men in their eighth decade (*15*). In dogs, the incidence of benign hyperplasia was estimated at about 80 percent in animals over 8 years of age (*10*), but it may occur in almost all senile dogs with functioning testes (*16*). However, the character of the prostatic enlargement in dogs is very different from that in man and consists essentially of a diffuse cystic hyperplasia (*16*, *38*). Figure 12 illustrates this type, which contrasts sharply with hyperplasia in man (fig. 14).

The question of why the prostate of aging men and dogs should be a site of benign and malignant growths has stimulated much research and speculation (*10*, *12*, *13*, *15–21*). There is almost a consensus that endocrine factors are of prime importance, and this concept provides the rationale for therapy. However, there are many other factors. One of the most important questions is why benign hyperplasia in man occurs in the inner group of prostatic tubules, while carcinoma originates in the posterior (dorsal) or outer group.

GENERAL CHARACTERISTICS OF PROSTATE GLANDS

All prostates are tubulo-alveolar glands with a secretory epithelium lining the alveoli. Usually they also have a basement membrane resting on a stroma of connective tissue containing smooth muscle fibers that surround alveoli and ducts. This stroma is rich in blood vessels. Nerve endings of various types are found in the connective tissue and epithelium. At ejaculation the smooth muscle fibers contract under sympathetic stimulation, and the acini are partially emptied of their secretions.

The gross structure of the prostate varies from a disseminate or diffuse type in which the glandular acini remain in the urethral stroma to one in which the acini form an external "body," sometimes lobed, outside the urethra. Or the prostate may be a combination of both types. Text-figure 1 shows the disseminate type in the opossum and the compact body of the prostate surrounding the urethra in man and dog. In the bull there is an external body that is continuous with a disseminate region. A familiar example of a lobed prostate is that of the rat (fig. 4). Rabbits present still other variations (text-fig. 2).

The original criterion for the classification of an accessory gland as a prostate ("one who stands before") was apparently that the gland must lie on the urethra near the neck of the bladder and the ducts must open into the prostatic urethra at the level of the verumontanum, or craniad and caudad to this level. The acini of the diffuse prostate of the opossum were classified as urethral glands and not recognized as a prostate (*22*).

In some animals, this type of classification resulted in the grouping together of several discrete lobes or glandular regions that differed in the adult in anatomical position, histological structure, (figs. 1, 2, 3, and 5) and the chemical composition of the lobes and their secretions (*1*, *2*). The rat is such an example, but the original craniodorsal prostate lobe was renamed as the coagulating gland on the basis of function (*23*, *24*). The diffuse prostate of the opposum has three histologically distinguishable regions anteroposteriorly (*25*, *26*). Conversely, the prostates of the dog and man have a uniform histological structure within the gland, but there are regions differing so clearly in response to hormone stimulation that a dual nature of the tissue is exposed. Also, in man, as mentioned previously, the inner region is involved in benign hyperplasia and the outer, or posterior region, is the site of origin of carcinoma.

In the interpretation of comparative studies on the prostates of experimental animals it may be very important to know which lobes or regions are homologous, particularly in attempts to apply the results of animal experimentation to pathological problems in man. Fortunately the homologies can be traced through embryological study, since the relationships of the duct openings into the prostatic urethra remain in the adult in the same relative position as in the fetus. Homologous lobes or regions in different species do not necessarily have the same chemical functions (*1*) or histological structure (*2*). However, homology may, in some instances, provide a sound basis for understanding responses to hormones and thresholds of response.

SOME COMPARATIVE ASPECTS OF STRUCTURE AND DEVELOPMENT OF THE PROSTATE

Man

The prostate in adult men is a compact organ lying at the neck of the bladder and surrounding the urethra. The ejaculatory ducts pass through its substance and the whole is enclosed within a firm, fibrous capsule. The lateral surfaces are prominent and rounded. It is frequently described as having two lateral lobes and a median lobe, but internally there is no such pattern. The acini are distributed in the form of an external group ("prostatic glands proper") that almost completely surrounds an inner or periurethral region containing prostatic acini, mucosal glands, the prostatic utricle, and the prostatic urethra itself (fig. 13). However, when the ducts of the lobules are traced to their urethral openings, the prostate can be divided into five lobes: a dorsal or posterior lobe dorsal to the ejaculatory ducts with ducts that open into the dorsal wall of the urethra distal (caudad) to the verumontanum; a median or middle lobe between the ejaculatory ducts and the urethra with duct openings in the dorsal urethral wall proximal (craniad) to the verumontanum; two lateral lobes, the ducts of which empty on each side of the verumontanum in the prostatic furrows; a small ventral or anterior lobe (which may be absent or atrophic) with ventral duct openings (*27*). The histological structure is similar in all these lobes (*12*), but Huggins (*13*) was able to map the limits of the posterior lobe by its specific response to estrogen. In his usage, the anterior prostate would include lateral lobes, middle lobe, and ventral lobe.

The five lobes correspond exactly to the five discrete groups of epithelial cords that grow out from the entodermal lining of the urethra and urogenital sinus in the fetus and form the prostate (*28*). Figure 7 summarizing Lowsley's excellent study shows clearly the position of these primordia in relation to the prostatic utricle that opens on the colliculus seminalis in close relationship to the ejaculatory ducts.

Monkey (*Macaca mulatta*)

The prostate lies entirely on the dorsal aspect of the urethra at the neck of the bladder. There are two discrete lobes (*22, 29*). The cranial lobe has a lobulated surface and resembles grossly the seminal vesicles to which it is closely applied. It completely surrounds the ducts of the seminal vesicles and the proximal ends of the deferent ducts. The caudal lobe is compact and smooth and is located mainly dorsal to the urethra and the ejaculatory ducts. The ejaculatory ducts are formed at the cranial level of the caudal lobe and join the urethra at a caudal level.

Histologically, the two lobes differ markedly, and they also differ in function. van Wagenen (*29*) showed that the secretion of the cranial lobe coagulated the secretion of the seminal vesicles. Regarding homologies, it was suggested that the cranial lobe in the monkey might be homologous with the middle lobe in man and the caudal lobe homologous with the lateral lobes and posterior (dorsal) commissure in man. This was on the basis of anatomical position. The site of entrance of the ducts into the urethra was not described.

In a 96-day monkey fetus, both lobes of the prostate were well developed (*30*). The buds of the cranial or cephalic lobe surrounded the ejaculatory ducts and prostatic utricle dorsally, laterally, and, to some extent, ventrally. The course of the buds and their entrance into the urethra were not mentioned, but they must have joined the urethra craniad to the openings of the ejaculatory ducts. The epithelial cords of the caudal lobe were described as lying mainly below (caudad) the entrance of the ejaculatory ducts. A figure showed some of the buds clearly deriving from the prostatic furrows.

In tracing homologies for the macaque prostate it seems reasonable to propose that the caudal lobe may be homologous with the lateral lobes in man. The cranial lobe probably corresponds in the position of embryonic origin of the cords, with the middle lobe of man, but not in anatomical position of their peripheral ends (dorsal to the ejaculatory ducts). Homology of the cranial lobe with the coagulating glands of rats and mice (to be described) seems probable. The monkey has neither ventral nor, apparently, dorsal lobes such as are found in man.

Rat

The prostate gland of the rat is composed of discrete paired ventral lobes at the neck of the bladder and a dorsolateral group of prostatic acini and their ducts. The latter acini surround, dorsolaterally, the urethra, base of the bladder, seminal vesicles, and coagulating glands. They thus enclose the proximal part of the deferent ducts with their ampullary glands (fig. 4). This dorsolateral "lobe" can be dissected, with more or less success, into paired lateral lobes and paired dorsal lobes. The histological structure differs in the various lobes of the prostate and the coagulating glands (figs. 1, 2, 3, and 5), as does the chemical composition of the lobes and their secretions (*1*, *2*).

Recent studies have made these lobes of especial interest (*1*, *2*). There has been some confusion as to the demarcation of the dorsal from the lateral lobes and a conjecture that lateral- and dorsal-type epithelia might coexist in the same acinus (*31*) or that there might be dorsal, dorsolateral, and lateral glandular units (*32*).

The problem can be clarified somewhat by examination of the relationship of the prostatic ducts in the fetus. The embryonic development of the prostate in the rat and mouse has been studied extensively (*33*), but little, if any, attention has been given to defining the dorsal and the lateral lobes in the rat on the basis of their precise origin from the urethra. The tendency has been to group these primordia and to describe them as a dorsolateral lobe.

I have recently restudied the embryologic development of the rat prostate with respect to the origin of the dorsal and lateral lobes. In a 19½-day fetus two pairs of epithelial buds grew out from the dorsal tip of each prostatic sulcus, at a level far caudad to the openings of the ejaculatory ducts and prostatic utricle into the urogenital sinus. Two to three pairs of these buds grow craniad and at later fetal ages their peripheral tips lie dorsal to the ejaculatory ducts and prostate utricle and craniad to the level of the Müllerian tubercle (fig. 10). The origin of the epithelial cords and the position that their tips ultimately occupy correspond exactly to the cords of the dorsal (posterior) lobe of the prostate in man. At 5 days after birth (fig. 8) it is clear that from the point of view of origin of the primordia and the anatomical position of the tubules, the dorsal prostate of the rat is homologous with the dorsal (posterior) lobe in man (fig. 7).

The lateral lobes in the rat originate from the dorsal and lateral walls of the right and left prostatic sulci or furrows at about the level of the entrance of the ejaculatory ducts into the sinus (fig. 10). Some of the most dorsal buds enter just caudad to the buds of the coagulating glands (fig. 9) and thus proximal to the entrance of the ejaculatory ducts. The numerous lateral prostate cords grow craniad, and at 5 days after birth are in juxtaposition at their dorsal limits with the dorsal lobes from which they are not clearly demarcated. They extend laterally and ventrally down to the ventral lobes from which they are separated by a mesentery. These lobes correspond exactly to the lateral lobes in man and may be considered homologous with them.

Figure 8 shows the division of the lateral lobes into groups of lobules as the cords have branched. However, there is no sharp division between the buds of the lateral and dorsal lobes where they meet dorsally. The clue to the identity of specific buds is given by tracing them to their origin in the urogenital sinus or urethra. The openings of their future ducts into the urethra are relatively widely separated—the ducts of the dorsal lobes, caudad, and those of the lateral lobes, craniad. It seems unlikely that on an embryologic basis there could be any inclusion of prospective epithelium of the two lobes in the same cord of epithelial cells. Therefore it seems improbable that, postnatally, cells of the two histologically different epithelia would appear side by side in the same acinus because of mixed embryologic origin.

The ventral lobes of the rat prostate are derived from three or four pairs of buds symmetrically arranged on right and left (fig. 10). The first buds appear from the ventral wall of the urethra caudad to the level of the colliculus seminalis, but in older fetuses they are present at the level of the ejaculatory duct openings into the urogenital sinus. The site of origin of these epithelial cords in the rat is very different from the origin of the ventral lobe in man, in which the buds emerge from the ventral urethral wall far craniad to the prostatic utricle (fig. 7). The ventral lobes in rat and man do not appear to be homologous, at least from the evidence of the region of the urethra from which they develop.

The homology of the prostate of the female rat with the ventral prostate of the male has been established (*2*). Functional similarity was shown between these homologues when androgen administration to females caused secretion of citric acid in the prostate (*34*). The production of this substance is a characteristic of the male ventral prostate under androgenic stimulation (*1*). The histological similarity of the male and female glands is apparent in figures 5 and 6.

The coagulating glands of the rat arise from a single pair of buds (fig. 9) originating dorsally from the cranial ends of the prostatic furrows.

They are thus proximal to the colliculus seminalis and the ejaculatory duct openings. They grow cranially without branching until they reach the stroma adjacent to the seminal vesicles which are developing in the genital cord. The middle or median lobe of the prostate in man also originates craniad to the colliculus seminalis (*28*). The nine or 10 tubules of this lobe grow out from the dorsal wall of the urethra (fig. 7), and ultimately the largest ones, which are near the midline, open high on the verumontanum as it develops. These cords branch in the stroma surrounding the ejaculatory ducts and prostatic utricle. On an embryologic basis, the coagulating glands might be considered homologous with the middle lobe in man.

Mouse

The coagulating glands and prostate resemble in general those of the rat. The mouse, too, has paired coagulating glands and dorsal and ventral prostatic lobes, although the ventral lobes are relatively less well developed than in the rat. Lateral lobes are absent or only slightly developed.

Examination of a limited number of mouse fetuses established that the dorsal lobes originate exactly as in the rat: from two to three pairs of buds that develop caudad to the orifices of the ejaculatory ducts. A few lateral buds arise as in the rat but apparently do not continue their development postnatally to any extent. The bilobed ventral prostate, with its three pairs of ducts, and the coagulating glands are homologous with the same organs in rats.

Dog

The prostate is compact and relatively large. It completely surrounds the urethra at its junction with the neck of the bladder. A median dorsal furrow is present indicating right and left hemispheres (*35*, *36*).

The branching secretory acini and ducts radiate on all sides of the prostatic urethra (fig. 11) and resemble a series of pyramids of tissue, with the base at the periphery of the gland and the apex at the urethra (*37*). The histological structure is similar in all the acini but estrogen administration causes epithelial hyperplasia, metaplasia, and fibromuscular stimulation, which occurs mainly in the dorsal half of the gland (*38–40*). In addition, application of the periodic acid-leucofuchsin technique brought out differences between the dorsal and ventral regions (*41*). The intrinsic acinar blood supply is a well-developed capillary network surrounding the acini (*42*), separated only by a delicate basement membrane from the secretory epithelium.

Few studies are available on the embryology of the dog prostate. The buds appear at the beginning of the 6th week with a body length of 27 mm (*43*). Regnauld (*37*) described the stage of prostatic development in fetuses 60 to 80 mm long. A single pair of buds, present caudad to the openings of the ejaculatory ducts, grew dorsally from the dorsal urethral wall; in the middle of the prostate (presumably near the prostatic utricle) one pair of buds grew out from the dorsal wall and a second pair from the ventral. The dorsal buds were always better developed than the ventral. These three pairs of buds were symmetrical in relation to the anteroposterior and dorsoventral axes.

Zietzschmann and Krolling (*43*) showed a figure illustrating a section at the level of the colliculus seminalis in a 42-day fetus, 78 mm long. There appeared to be four pairs of symmetrical prostatic buds: one dorsal bud arising from the dorsal end of each prostatic furrow; two ventral buds, one on each side of the midline; and one pair of lateral buds on each side extending out from the lateral extremities of the urethra. The buds thus radiate in all directions. There are indications of several other buds which join at another level. The pyramids of tissue (figs. 11 and 12) presumably represent the branching acinar systems derived from these primordia in the fetus.

It is impossible to trace homologies definitely on such scanty evidence. But it is tempting to interpret the pair of buds that arise dorsally and far caudad as the primordia of a dorsal prostate (region) that might be homologous with the dorsal prostate of man. There is some support for this interpretation in the observations of Berg (*40*). He reported that a specific dorsocranial region in the puppy and adult dog responded much less markedly to estrogen than the remainder of the dorsal lobules. It is possible that the cranial dorsal buds, the lateral buds, and the ventral buds are homologous, respectively, with middle, lateral, and ventral prostatic lobes of man. The pronounced estrogenic response of the dorsal lobules (with the exception of a dorsocranial region) may be significant in this regard and indicate homology with the middle lobe of man. Further embryologic study might establish this point.

SOME COMPARATIVE ASPECTS OF PROSTATIC RESPONSES TO HORMONES

Since normal growth, differentiation, and function have an endocrine background, it is essential to study the responsiveness of the prostate to hormones under various conditions. This is all the more necessary because, in the etiology of benign hyperplasia and prostatic carcinoma, hormones, among other factors, play some role. It is known that androgenic and estrogenic hormones are present in the hormone milieu of the male fetus as well as postnatally through adult life and senescence. Estrogen levels or androgen-estrogen ratios have been suggested as important factors in development of pathologies.

Man

It is well known that sex hormones normally affect the male fetus. At birth the scrotum is swollen and turgid, the breasts are enlarged and colostrum is secreted, and the prostate is hypertrophied (*45*). There are evidences of both androgenic and estrogenic stimulation.

In the fetal prostate, squamous metaplasia and focal hyperplasia of the acini have long been recognized (*44–48*). The metaplastic changes, typical of estrogenic action, involve the epithelium of the acini, the prostatic utricle, and the dorsal wall of the urethra. The focal hyperplasia results in the production in the acini of a tall columnar epithelium of an adult type, which is characteristic of androgenic stimulation. After birth, the metaplasia and hyperplasia retrogress relatively quickly, and not until puberty do the acini again have a tall secretory epithelium.

Andrews (*48*) attempted to relate these changes to the specific lobes of the prostate in the fetus and newborn child, ranging in age from the 24th week of fetal life to 1 month after birth. In 47 prostates squamous metaplasia occurred in the dorsal (posterior) lobe in 16; in the lateral lobes in 38; in the middle lobe in 38; and in the ventral lobe in 22. Localization of metaplasia and hyperplasia was of interest. Metaplasia was slight in the dorsal lobe and appeared caudally at the origin of the tubules from the urethra, or peripherally at their cranial tips. The lateral lobes showed metaplasia in the proximal regions of the tubules close to their origin from the prostatic furrows. Only occasionally was there a slight peripheral change. Extensive metaplastic changes occurred in the middle lobe and were distributed along the tubules all the way from proximal to distal ends. The buds originating near the opening of the ejaculatory ducts were most affected. In the ventral lobe, metaplasia was slight and appeared only near the urethral ends of the tubules.

Focal hyperplasia, with patches of an adult type of epithelium, was usually found in the dorsal and lateral lobes and only rarely in the middle and ventral lobes.

From this study it may be tentatively concluded that: The lateral and middle lobes are more responsive to estrogens; the dorsal and lateral lobes are more sensitive to androgenic stimulation; and the ventral lobe is relatively insensitive. In terms of the individual lobes, the dorsal is predominantly androgen-sensitive; the middle, markedly estrogen-sensitive; the lateral, responsive to both types of hormones; and the ventral, only slightly responsive to androgens and estrogens.

This hormone sensitivity in the fetus may be of crucial significance in the normal development of the prostate. Moore (*10*) observed that the structure of the prostate in pseudohermaphrodites depends upon the sex of the gonad. If there are ovaries, only the middle and lateral lobes are represented. If testes are present, the whole prostate, including the dorsal (posterior) lobe, is found.

Estrogens have been administered to newborn babies and adult men. Such treatment of the newborn for 7 weeks and 7½ months prevented the normal retrogressive changes in the prostate (*49*). The dorsal urethral epithelium, prostatic utricle, and uppermost prostatic collecting ducts were metaplastic. These ducts lay in the so-called utricular bed and were in all probability mainly those of the middle lobe.

Estrogen treatment of adult men caused squamous metaplasia of the urethra and prostatic ducts (*50*). However, a particularly enlightening study by Huggins (*13*) showed that estrogen administration to an adult caused much more marked degenerative changes in the anterior region than in the posterior (dorsal). Huggins' classification of the anterior region includes Lowsley's categories of lateral, middle, and ventral lobes. Interestingly enough, this estrogen sensitivity of the anterior region agrees with the observations of Andrews in the fetus (*48*).

It remains to be demonstrated whether there is any specific relationship between hormone sensitivity of the lobes and the fact that carcinoma is mainly restricted to the posterior lobe and benign hyperplasia to the middle and lateral lobes. In the aging process in the human prostate (*12*, *51*, *52*), responses to hormones may change qualitatively or quantitatively. An increase in hormone sensitivity occurs at puberty, at the opposite end of the life cycle (*53*).

Monkey (*Macaca Mulatta*)

Some stimulating effects of prenatal sex hormones are evident in certain organs in the near-term fetus and in the newborn. The testes descend (they ascend again soon after birth), scrotal skin is reddened and swollen, and the nipples are erect and pink (*54*, *55*). However, no metaplasia or hyperplasia of the fetal prostate was reported (*30*). When testosterone propionate was administered to pregnant females, the prostatic lobes of male fetuses were somewhat enlarged, which indicated androgen responsiveness (*30*).

The administration of estrogen postnatally enlarged the prostate (*56*). Fibromuscular tissue was relatively increased as compared with the glandular tissue, and this reaction was more marked in the cranial lobe. The glandular epithelium remained single-layered, but the epithelium of the prostatic utricle and the urethra over the colliculus seminalis underwent squamous metaplasia.

Zuckerman (*57*) injected estrogen into rhesus monkeys for a year. He reported that the prostates were greatly enlarged. This was mainly due to fibromuscular growth in the utricular bed, but the glandular epithelium was essentially unchanged. Hyperplasia and metaplasia occurred only in the epithelium of the urethra, prostatic utricle, the collecting ducts of the prostate, and the terminal ends of the ejaculatory ducts. In discussing his results and those of others, he stressed the unresponsiveness of the prostate of monkeys to estrogens.

Rat and Mouse

There is ample evidence based on fetal castration that the prostate glands of these rodents are normally responding to androgens prenatally (*58*). In fact, androgenic hormones from fetal testes are essential for the normal formation of the prostatic primordia. The results of culturing isolated reproductive tracts of fetal rats further emphasized this androgen dependence (*59*, *60*).

There are no observable estrogenic effects in the prostate in normal fetal development. The addition of estrogen to the culture medium of fetal rat reproductive tracts did not produce any prostatic changes (*60*). Administration of estrogen to pregnant female rats and mice suppressed the development of prostatic buds in fetuses (*58*, *61*).

There are marked species differences in the responses of rats and mice to estrogen given postnatally (*2*, *18*, *62*). In rats, fibromuscular hyperplasia and epithelial metaplasia in the ducts and acini occurred in the coagulating glands. Similar changes appeared in the dorsal prostate but to a lesser degree. The lateral and ventral prostates showed only slight effects, and the epithelium was not involved.

In contrast, mouse prostates were highly responsive to estrogens. Fibromuscular hyperplasia and epithelial metaplasia were reported in coagulating glands and dorsal and ventral prostate lobes. The changes were most marked in the coagulating glands.

In both species the coagulating glands were more reactive than prostatic lobes. In both species, the duct epithelium was more responsive than that of the acini. The first responses occurred in the ducts at their urethral ends and progressed peripherally into the acini.

The relatively high degree of responsiveness of the coagulating glands to estrogen is reminiscent of the reactivity of the middle lobe of the prostate in man. The two may be homologous on embryologic grounds. Such a homology was suggested because of anatomical position (*63*). In the monkey, the cranial lobe, which is functionally a coagulating gland, was more responsive to estrogen than the caudal lobe (*56*). Homology of the monkey cranial lobe with the rodent coagulating gland is probable, but the relative unresponsiveness in the monkey emphasizes the importance of species differences.

Observations suggest that senile involution may occur in the rat and mouse prostate (*51*). A cytochemical change in the dorsal and ventral lobes of 2-year-old rats was reported (*64*). Harkin observed modifications in ultrastructure of the rat prostate with advancing age (*65*).

Dog

No reports were found in the literature on androgenic or estrogenic effects in the prostate of the fetus during normal development.

When estrogen was administered to newborn puppies, the prostate was much enlarged, particularly in the dorsal region (*40*). There was dilatation of the acini and squamous metaplasia of the epithelium, which was less marked in a region that was dorsal and cranial to the colliculus seminalis. In older dogs, estrogen caused epithelial metaplasia in prostatic ducts and acini—particularly in dorsal acini—and in the urethra and prostatic utricle (*38–40*). Similar changes were observed in prostates of dogs with estrogen-secreting Sertoli cell tumors of the testis (*11*, *38*, *66*).

In cystic hyperplasia in senile dogs, estrogen treatment at lower dosage levels reduced the size of the prostate and resulted in atrophy of the gland

(*39*). Castration had the same effect, and the dependence of cystic hyperplasia on testicular androgens in the aging dog is clear. In dogs with estrogen-secreting testicular tumors cystic hyperplasia did not appear (*66*).

Senile changes in the prostate, other than cystic hyperplasia, are not evident. With aging, the capillary network around the acini becomes increasingly complex as hyperplasia progresses (*42*). The great increase in vascularity in senile hyperplasia was noted by Huggins and Clark (*39*).

SUMMARY

Some of the available information on the embryologic development of the prostate glands of man, monkey, rat, mouse, and dog was reviewed and studied. On this evidence, certain tentative homologies were proposed between prostatic lobes or regions in the several species. The five "lobes" of the prostate of man were accepted as organic entities, as proposed by Lowsley from his study of the fetus. The posterior (dorsal) lobe of man may be homologous with the dorsal lobe of the rat and mouse and, possibly, with a dorsocranial region in the dog prostate. The middle lobe of the human prostate may be homologous with the cranial lobe of the monkey and the coagulating glands of rats and mice. The lateral lobes of man, the caudal lobe of the monkey, and the lateral lobes of the rat and mouse prostate appear homologous. The ventral prostatic lobe of man does not seem homologous with the ventral lobes of the rat and mouse.

The sensitivity of the prostatic lobes to hormone stimulation, more particularly to estrogen, showed that in some instances homologous lobes gave somewhat similar responses. This was true for the middle lobe in man, the cranial lobe of the monkey, and the coagulating glands of the rat and mouse. These glands in the monkey, rat, and mouse were functionally equivalent in the production of a secretion which coagulates the secretion of their seminal vesicles. Furthermore, cross-coagulation between species has been demonstrated (*29*). However, homology of the lobes or glands in different species is not necessarily correlated with similar hormone responsiveness or with the specific chemical composition of the glands and their secretions. The relation of estrogen sensitivity to the embryonic origin of the epithelium of the urogenital sinus has been discussed and argued (*67–70*).

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Plate 1

Accessory reproductive glands of the rat.

Figure 1.—Coagulating gland. × 500

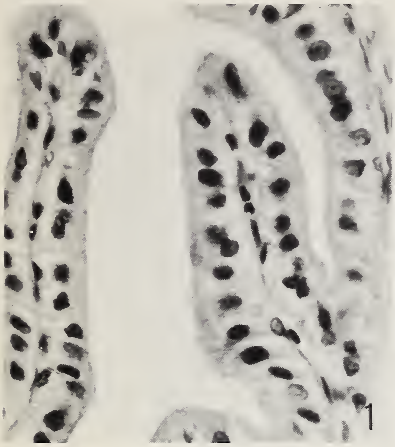
Figure 2.—Dorsal prostate. × 500

Figure 3.—Lateral prostate. × 500

Figure 4.—Ventral aspect of male accessory glands. Right coagulating gland has been freed from the seminal vesicle to show relation of the ducts. Bulbourethral glands are not shown. Key: A, ampullary gland; BL, bladder; D, ductus deferens; DPR, dorsal prostate; LPR, lateral prostate; UR, urethra; VPR, ventral prostate.

Figure 5.—Ventral prostate. × 500

Figure 6.—Prostate gland of a 28-day-old female. × 250



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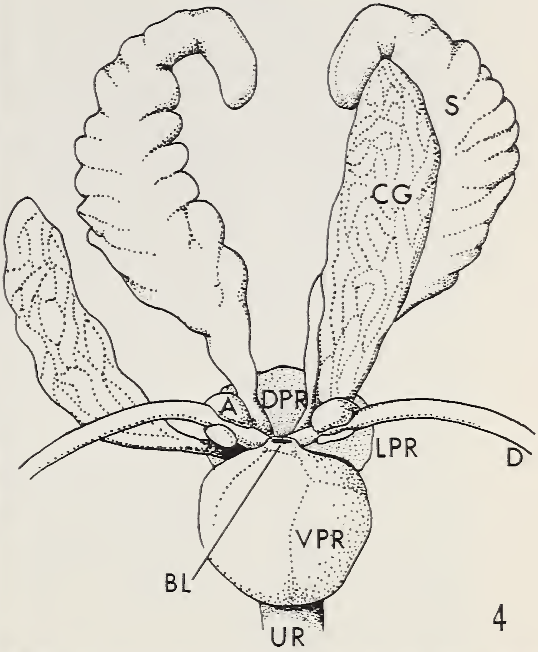
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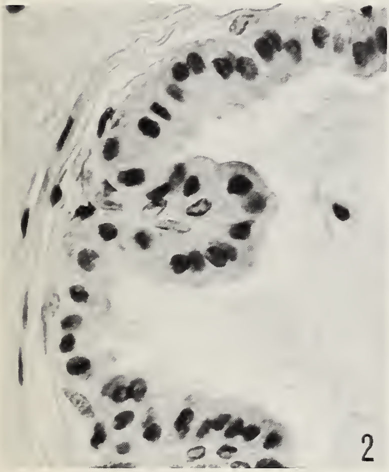

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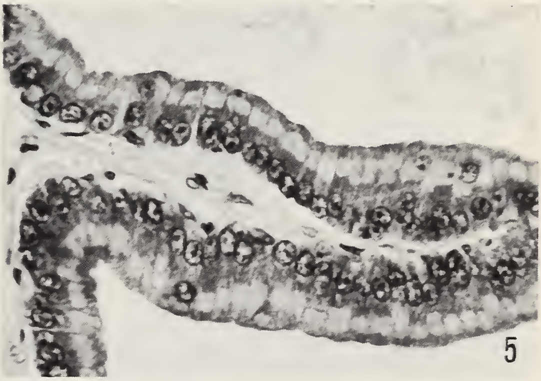

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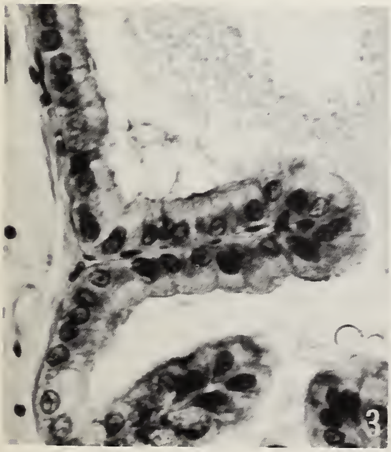

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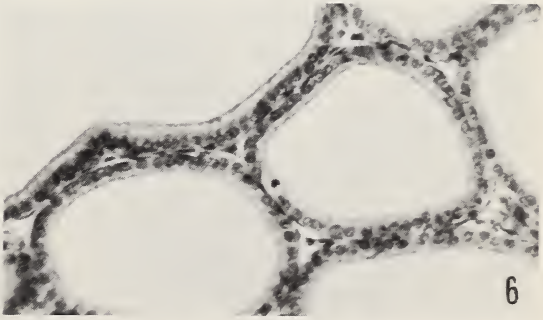

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Plate 2

Figure 7.—Reconstruction of the prostate gland of the human fetus.

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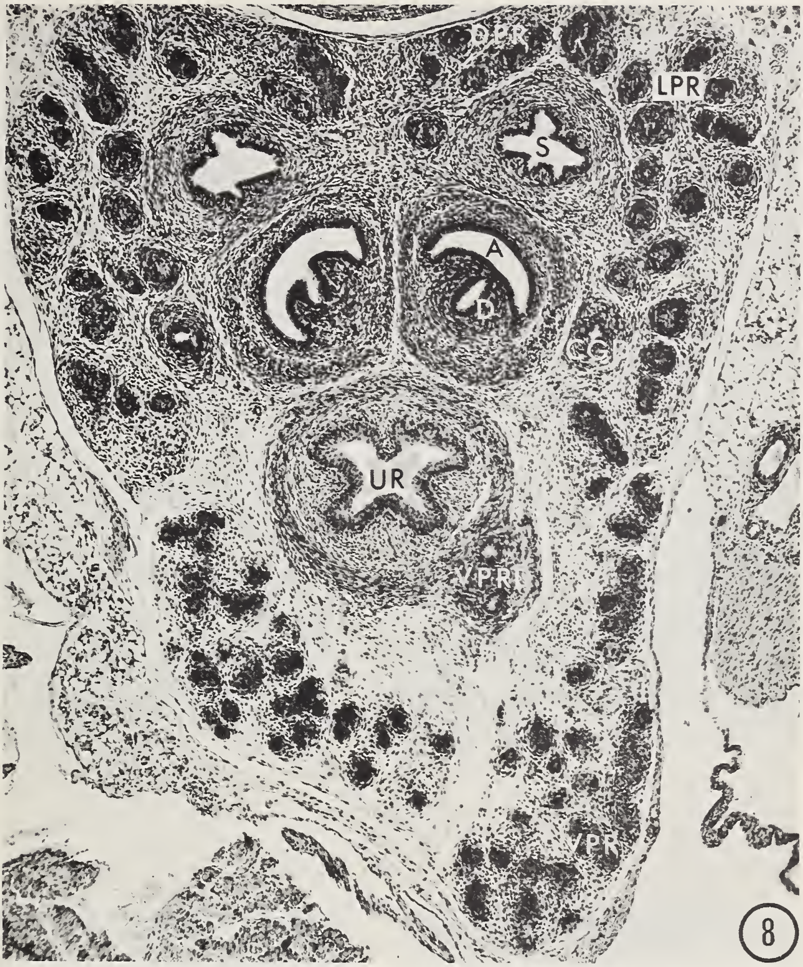
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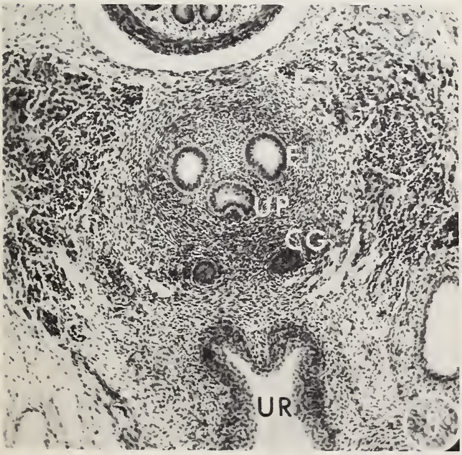
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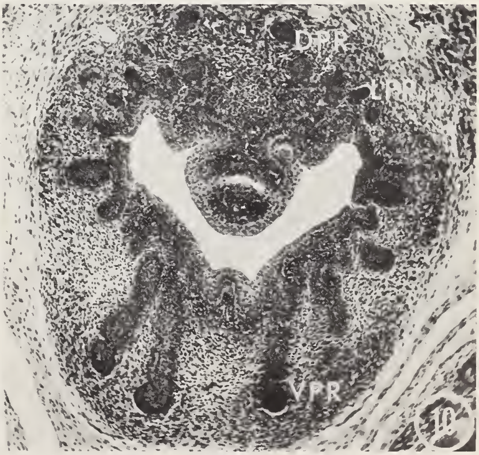
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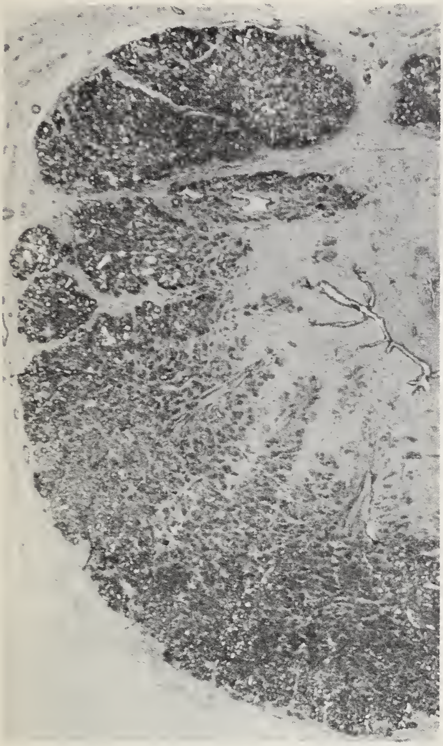
PLATE 4

FIGURE 11.—Normal prostate—dog. [Reprinted from the *Journal of Pathology and Bacteriology* (*38*).]

FIGURE 12.—Prostate with cystic hyperplasia—dog. [Reprinted from the *Journal of Pathology and Bacteriology* (*38*).]

FIGURE 13.—Normal prostate—man. [Reprinted from the *Annals of the Royal College of Surgeons of England* (*15*).]

FIGURE 14.—Prostate with benign hyperplasia—man. [Reprinted from the *Annals of the Royal College of Surgeons of England* (*15*).]



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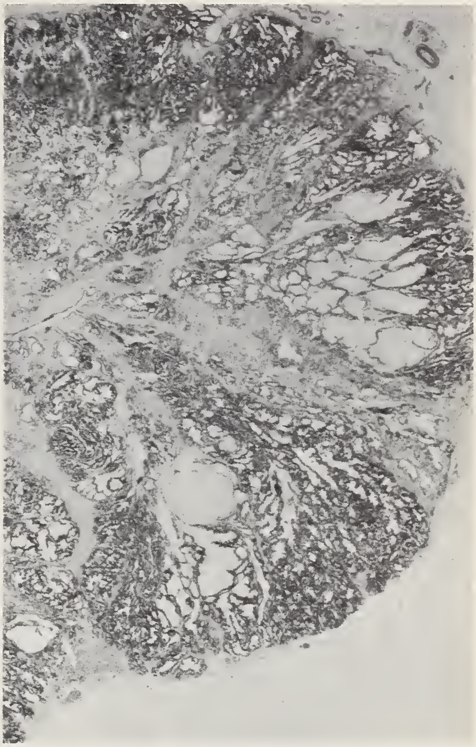

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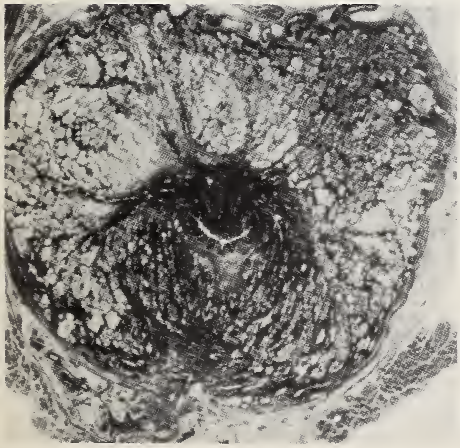

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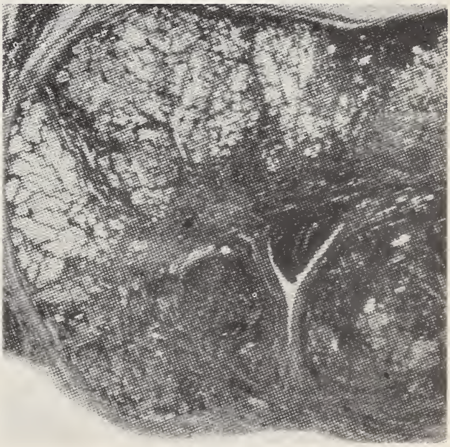

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DISCUSSION

Dr. Mann: Dr. Price in her paper—notable for characteristic clarity of presentation—touched, among others, on the fundamental problem of homologies between male accessory organs of different species. It is of considerable interest that glandular structures, which we know from embryological and other morphological studies to be closely related and homologous, often perform quite different functions so far as the products of their metabolism are concerned. Two related species, the rat and mouse, may be cited as examples. Whereas in the rat the seminal vesicle produces citric acid but no fructose and the coagulating gland only fructose but no citric acid, there is no such distinction between the two glands in the mouse. The reason for such discrepancies between morphological and biochemical characteristics still remains to be explored.

We should not forget, however, that a similar problem is posed by observations made on other organs in the animal body. It is well known, for example, that there are profound differences in the liver metabolism of various mammals, particularly as regards the purine metabolism and the occurrence of such enzymes as xanthine oxidase. Another possibility is that two accessory organs, structurally homologous, may be quite different in "permeability" to extraneous substrates, reaching the glands via blood. We have shown that when ergothioneine (the betaine of thiolhistidine) is fed to the boar in the form of a radioactive compound, this substance is secreted unchanged in the seminal vesicle secretion. Ergothioneine is also, however, a normal, physiological constituent of the boar vesicular secretion. There is obviously, no strict barrier in the accessory organs to substances that reach these glands via blood.

Dr. Price: I am glad that Dr. Mann has stressed this very important point.

Dr. Brendler: Dr. Price, did you say that the monkey prostate was comparatively insensitive to hormones? Was this in the fetus, in the adult, or in both?

Dr. Price: I said that the prostate gland of the monkey is relatively unresponsive to estrogens postnatally, particularly as compared with the rodent. There is no evidence that the fetal gland is normally responding to estrogens prenatally. This is in contrast to the estrogen responsiveness of the fetal prostate of man.

Dr. Brendler: And to castration?

Dr. Price: I do not know how rapidly the monkey prostate regresses after castration.

Dr. Kochakian: Dr. Price, you talked about testosterone and estrogen stimulating the histological changes in the prostate. Do you have any comments in respect to the combination of the two? We have always been intrigued by the fact that the combination of testosterone and estrogen results in the inhibition of accessory sex organs but not of the kidney. Do you have anything to add to this?

Dr. Price: In the human fetus, androgens and estrogens are present simultaneously and act on the developing prostate. Here the androgens do not prevent the metaplastic effect of the estrogens. In the rodent prostate postnatally, androgen administration may prevent the pathological effects of estrogen.

Dr. Kochakian: What is the histological picture when they are balancing each other?

Dr. Price: At the proper dosage levels androgen completely "protects" the prostate from estrogen effects.

Dr. Kochakian: Would the net result be an androgenic type of response with no indications of the estrogen effect?

Dr. Price: That would depend upon the dosage, the gland, and the species of animal. It is possible to get both types of effects simultaneously, but usually androgen will protect the epithelium from the metaplastic effect of the estrogen.

Dr. Kochakian: I was wondering whether the term "protective" is the right designation, or whether, when there is an improper balance, something synergistic or supplementary is going on.

Dr. Price: The results on the rodent prostate of administration of estrogens to intact males, or androgens and estrogens to castrated males, have been described as competitive, protective, or in some cases synergistic. An example of two types of effect was shown by Freud and his collaborators. They reported fibromuscular growth in the seminal vesicles of castrated rats treated with estrogen and also stratification in the epithelium of the ducts of the lateral prostate. When androgen was administered simultaneously with the estrogen, fibromuscular hypertrophy was increased but the epithelial changes in the prostatic ducts were prevented.

Dr. Huggins: One speaks of neutralization of androgenic effects in the prostate by estrogens, but this is a misnomer. In the castrated dog, when rather large doses of testosterone (10 mg/day) and diethylstilbestrol (1 mg/day) are given, the prostate grows very large. The characteristic effect of testosterone on the prostate is formation of tall columnar secretory epithelium. One recognizes the androgenic substances in this way. The characteristic effect of estrogens is squamous metaplasia of prostatic epithelium. In the castrated dog, whose prostate to begin with is small, one finds a very remarkable thing when testosterone and diethylstilbestrol in combination are given for, let us say, 30 days. In the anterior part of the prostate there are androgenic cells exclusively, without any sign of estrogenic stimulation. In the posterior part of the prostate one finds squamous epithelium, without any sign of effect of testosterone. In the urethra, squamous metaplasia characteristic of the estrogen is found. In other words, the prostate does not remain small but undergoes tremendous growth. Part of the gland shows response to testosterone and the other shows only an estrogenic response.

Dr. Kochakian: What happens, Dr. Huggins, when you give a large dose of estrogen? Is there an inhibition of the over-all size of the prostate?

Dr. Huggins: Not at all. For a dog 1 mg per day of diethylstilbestrol is a large dose. If one gives 10 or 100 mg of this phenolic estrogen the same effect is obtained. One cannot wipe out the characteristic effect of testosterone on the responsive glands with any amount of estrogen. Similarly one cannot wipe out the estrogenic effect with any amount of testosterone; areas of the prostate respond, respectively, to testosterone or phenolic estrogens with a preferential, characteristic response.

Dr. Nadel: Have there been any attempts to affect the gland locally by judicious administration of single or combination administration of these drugs?

Dr. Grayhack: Yes. We have implanted estrogen pellets, both diethylstilbestrol and estradiol, into the dog prostate and the rat prostate. We have implanted combinations of estrogens and testosterone. With the local implantation of the estrogen, we have seen massive squamous metaplasia in the dog. With the combination we have seen squamous metaplasia closest to the pellet and then the hyperplasia distal to this. We have found no evidence of inhibition of weight increase of one lobe of the ventral prostate by implantation of a pellet of estrogen in the presence of exogenous systemic androgen. We noted evidence of a synergistic effect with regard to the citric acid content of the lateral lobe when systemic estrogen and androgen were administered to the rat.

HISTOCHEMISTRY OF SOME ASPECTS OF PROSTATE PATHOLOGY[1, 2]

DAVID BRANDES and G. H. BOURNE, Departments of Pathology, Johns Hopkins University School of Medicine and Baltimore City Hospitals, Baltimore, Maryland, and Department of Anatomy, Emory University, Atlanta, Georgia

THE prostate is a composite organ and biochemical studies provide only an "average" figure for the whole organ: They do not tell whether compounds estimated are in the acinar cells or the stroma. It seemed appropriate therefore to make a histochemical study of the prostate under certain physiological and pathological conditions to assess the histological and cytological changes in distribution of various compounds and enzymes which occur under these conditions.

The substances studied were alkaline and acid phosphatase, lipase, α-esterase, succinic dehydrogenase, xanthine oxidase, 5-nucleotidase, protein-bound sulfydryl groups, and lipides (Sudan black method). In addition, preparations were made of the Golgi apparatus by the Da Fano method. The techniques used for the substances listed are given by Brandes and Bourne (*1*).

NORMAL PROSTATE

In the prostate of the normal mouse all three lobes showed a positive alkaline phosphatase reaction (fig. 1) in the basement membranes of the acini and in the wall of the small vessels. The stromal distribution of phosphatase in the accessory glands of the male has been recorded by other authors (*2, 3*). Acid phosphatase gave a positive reaction almost exclusively in the epithelium in all lobes. With Gomori's method (fig. 2), the nuclei gave a moderate reaction, but the greatest activity was in the region of the Golgi apparatus. Bourne (*4*) first recorded the localization of phosphatases in the Golgi region in gut epithelial cells by the alkaline phosphatase reaction. Acid phosphatase in the Golgi region of the epithelial cells of various organs has been recorded by Deane and Dempsey (*5*). However, recent investigations with the azo-dye method (fig. 3) showed the reaction in the form of small granules evenly distributed

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962, by Dr. G. H. Bourne.

[2] Part of this work was supported by grants C-5425, National Cancer Institute, and H-4178, National Heart Institute, National Institutes of Health, Public Health Service.

throughout the cytoplasm of the epithelial cells; the nuclei remained negative.

The periodic acid-Schiff (PAS) reaction for mucopolysaccharides gave a positive reaction in the basement membranes and the endothelium of capillaries; the epithelial cells also showed positive material in the cytoplasm (fig. 4). In most cells this material was aggregated near the free border.

The lipase reaction in the mouse prostate was negative, and the α-esterase reaction was positive only in the median lobe. The succinic dehydrogenase reaction was positive in the epithelial cells, and the xanthine oxidase reaction showed a few positive cytoplasmic granules, some of which resembled mitochondria. SH and SS groups were demonstrated in the basement membranes of the acini. There was a moderate reaction in the ventral lobe; in the anterior lobe it was stronger. In the epithelial cells of the three lobes, Sudan black preparations showed a positive reaction which was localized mainly in the Golgi region and resembled the Golgi apparatus as demonstrated by silver techniques (fig. 5).

EFFECTS OF CASTRATION AND SEX HORMONES

Castration and sex hormone administration are known to cause cytological changes in the prostate of the rat [*see* Moore, Price, and Gallagher, 1930 (*6*)]. In 1947 Horning (*7*) showed that castration also affects the Golgi apparatus in the mouse prostate. We found that after castration there was hyperplasia of the fibromuscular stroma which was moderate 8 days after castration but increased with the length of the period following castration. The epithelial cells lining the acini proliferated into several layers without, however, showing any squamous metaplasia. These changes were more pronounced in the anterior lobe than in the other lobes.

Six to 8 days after castration the PAS reaction (fig. 6) greatly decreased in the epithelium and increased in the basement membrane. The acid phosphatase reaction became less intense and consisted of discrete granules instead of the Golgi-like bodies seen in the normal mouse.

The alkaline phosphatase reaction (fig. 7) in the basement membrane showed considerable discontinuity and loss of intensity, or became completely negative.

Twenty-one to 22 days after castration these changes became accentuated. The PAS reaction was almost completely negative in the epithelium and increased in the basement membrane. The acid phosphatase reaction in the Golgi region was limited to very scarce granules (fig. 8). The alkaline phosphatase reaction completely disappeared from the basement membrane of most acini, but the sheaths of the smooth muscle fibers gave a positive reaction (fig. 9). The Golgi apparatus failed to become impregnated with silver in most of the epithelial cells (fig. 10).

The histochemical effects produced in the prostate by castration can also be obtained by the implantation of diethylstilbestrol. Biochemical estimations made on the prostate in castrated animals did not give any hint of a change of localization of enzymes. Thus in alkaline phosphatase preparations the decrease in enzyme in the basement membrane, which must be quite important from the point of view of cell physiology, has been compensated for by increase in the strength of the reaction in the sheaths of the hyperplastic smooth muscle fibers of the stroma developing after castration.

Following implantation of testosterone, or removal of diethylstilbestrol, the PAS reaction of the positive cytoplasmic granules and increased reaction in the basement membrane gradually returned to normal. Acid phosphatase activity in the epithelium regained its normal aspect and showed a strong reaction in the form of a compact body in the Golgi region (fig. 12).

The alkaline phosphatase reaction returned rapidly to normal and appeared in the basement membrane that again became an uninterrupted layer surrounding the acini (fig. 11).

The normal aspect of the Golgi apparatus was quickly attained following treatment with testosterone, the apparatus again appearing as a compact network in the supranuclear region.

HOMOGRAFT EXPERIMENTS

Pieces of anterior and ventral lobes of the prostate were implanted subcutaneously in the right and left axillary regions of Strong A male mice, 4 to 5 months old. These were actually homografts. In some, the prostatic tissue was impregnated with a few crystals of 3-methylcholanthrene before implantation, according to the method described by Horning (*8*). The animals bearing the grafts were killed at various times from 1 to 60 days.

Three weeks after the untreated grafts had been implanted, the prostatic tissue resembled the normal. The inflammatory reaction that appeared early in the stroma had disappeared and there was a general increase in connective tissue represented by the large amounts of ground substance, reticulin fibrils, and proliferating fibroblasts, some of which contained PAS-positive granules. The differences in vitality between the peripheral and central parts of the graft were less striking than in the earlier period of grafting.

The epithelial cells of both peripheral and central parts of the grafts showed abundant PAS-positive material. The acid phosphatase reaction that had disappeared in the beginning from the Golgi region reappeared, and the alkaline phosphatase reaction was again present at 3 weeks in the basement membrane as a sharp, uninterrupted line surrounding the acini. At this stage the lumina of the acini were filled with secretion. From the 4th week onward the grafted tissue appeared histologically and histochemically similar to the normal prostate *in situ*. After some time, however, the acini tended to become distended with secretion and there was resultant flattening of the epithelial cells in the grafts of the anterior lobe; grafts of the ventral lobe also showed similar changes but there was a difference in reaction. The normal histochemical picture did not return even after 7 weeks, and there was little sign, even at this time, of any return of secretion in the lumen.

All grafts treated with 3-methylcholanthrene, which became vascularized and survived, showed squamous cell metaplasia. There was a proliferation of the acinar epithelium showing a stratification of several layers instead of the usual single layer of columnar cells. These proliferating cells, which finally obliterated the lumina of the acini, showed keratinization and went on to form large masses of keratin. During this process marked changes in the histochemical pattern of the acini occurred. First there was a gradual disappearance of the intercellular and intracellular PAS-positive material, and the basement membrane became thicker and remained strongly positive. The acid phosphatase reaction in the Golgi region became feeble, but the keratohyalin granules, as they developed, appeared to be positive for acid phosphatase (fig. 13). The alkaline phosphatase, normally localized in the basement membrane, disappeared early in the process of squamous cell metaplasia. Obviously there were some fundamental histochemical changes presumably associated with the change of metabolism of these cells, as they began to produce keratin instead of various secretory products. Some grafts underwent malignant transformation and squamous cell carcinomas appeared. In these the acid and alkaline phosphatase reactions completely disappeared, but the basement membrane surrounding the epithelial core still gave a strong positive PAS reaction.

The significance of preparations in which acid phosphatase is localized in the Golgi apparatus is not known, but the Golgi apparatus has a large phospholipide component, and the phospholipide content of seminal fluid is dependent on hormonal stimulation. Phospholipides in the ventral prostate of the rat were decreased after castration but restored after implantation with testosterone. In the human prostate phospholipides constitute more than 50 percent of the total lipides of the glands (*9*). It has been suggested in the past that the Golgi region is connected with phospholipide synthesis; possibly acid phosphatase is concerned with this process.

Generally speaking we found considerable similarity between the effects of castration and those of estrogens on histochemical patterns. However, some differences occurred in the basement membrane where alkaline phosphatase and PAS-positive material decreased in castrated mice, whereas in diethylstilbestrol-treated animals only the alkaline phosphatase reaction decreased and the PAS reaction became stronger. This also occurred in the grafts treated with 3-methylcholanthrene.

We described this work in a paper in 1954 (*10*), and we were among the first to demonstrate the relationship between hormones (in this instance the male sex hormone) and the intracellular distribution of enzymes.

Many papers have since appeared in the literature, confirming that intracellular enzyme distribution is dependent on hormonal control. While the histochemical results we have demonstrated in squamous cell carcinoma and squamous metaplasia of the prostate are interesting, it is unfortunate that we were unable to obtain any glandular carcinomas, since this would have given us a more direct relationship between the normal glandular cell and the glandular cell in a carcinomatous state. The changes that we observed with squamous cell carcinoma may be due more to the actual change in character of the cell than to the fact that it became malignant.

HUMAN GLANDULAR CARCINOMA

One of our next studies was concerned with the histochemistry of human glandular carcinoma, in which changes from the normal were observed. In the human prostate, some histochemical reactions, especially for acid and alkaline phosphatases, were investigated in normal and carcinomatous prostates by Fergusson and Pagel (*11*), Fergusson (*12*), and Downey (*13*).

The current observations were reported by Brandes and Bourne in 1956 (*1*). This study was based on 13 pieces of material obtained from 3 carcinomas, 5 adenomas, and 5 normal glands. The following types of histochemical preparation were carried out: PAS reaction, acid and alkaline phosphatases, adenosine-5-monophosphate, α-naphthyl esterase, and lipase. Some of the preparations demonstrated fat by the use of Sudan IV and Sudan black. Some frozen sections of fresh tissue were also used to show cholinesterase and succinic dehydrogenase. SH and SS groups were also demonstrated in other preparations.

The PAS reaction in the normal glands was positive in smooth muscle fibers, blood vessel endothelium, and basement membranes of acini, fine granules being distributed in the cytoplasm of epithelial cells, especially in the supranuclear region. Neither amylase nor saliva affected the reaction, which presumably therefore was indicative of mucopolysaccharide. In adenomas there was no change in this reaction, but in carcinomas it resembled the normal; the less differentiated parts showed a diminution of reaction in the basement membranes of the acini.

α-Naphthyl esterase (fig. 14) showed a positive reaction in the cytoplasm of the epithelial cells and was accumulated mainly in the supranuclear and basal parts of the cell. Both adenomas and carcinomas showed a diminished reaction for this enzyme (fig. 15).

Acid phosphatase (fig. 16) indicated a positive reaction in the form of discrete cytoplasmic granules that were most intense in the ventral part of the gland. Occasionally, the secretion in the lumina was positive. No change could be observed in the adenoma or carcinoma.

In Sudan IV and Sudan black preparations of normal glands, fat droplets were present in the supranuclear and basal parts of the cytoplasm; there was no change in the adenomas. But in many acini of prostatic carcinoma there was a marked increase in the fat reaction in the epithelium.

5-Nucleotidase showed a positive reaction in the nucleoli and other granular material in the nucleus of both stromal and epithelial cells. There was no apparent change in the adenomas or the carcinomas (fig. 17).

The alkaline phosphatase (fig. 18) was positive only in the wall of small blood vessels. Contrary to the reaction in mouse and rat prostates, the basement membranes remained negative for this enzyme. No change could be seen either in the adenomas or carcinomas.

Lipase was negative in normal tissues, adenomas, and carcinomas.

Cholinesterase was investigated only in the normal gland, and a positive reaction was obtained in the smooth muscle fibers and the periacinus connective tissue. The SH and SS groups were positive in the smooth muscle fibers and moderately strong in the epithelial cells, and this reaction was slightly increased in the adenomatous nodules in adenomas. It was not investigated in carcinomas.

The succinic dehydrogenase reaction was moderately to strongly positive throughout the gland and there was a fine granular reaction in the epithelial cells. The reaction was slightly stronger in the adenomatous nodules of adenomas but was not investigated in carcinomas.

No detectable change could be found in reactions of normal and adenomatous prostates when subjected to PAS, Sudan black, acid and alkaline phosphatases, and 5-nucleotidase reactions. Although a decrease of the esterase reaction in prostatic adenomas and carcinomas was reported in our previous study, the application of improved histochemical techniques does not confirm that result. For various reasons we were unable to test the succinic dehydrogenase, sulfydryl, and disulfide reactions on carcinomatous material.

Other points to be noted in the carcinomatous material are the reduction in PAS reaction and the increase of sudanophil fat in some of the epithelial cells. Although some minor changes were reported in the distribution of acid phosphatase in prostatic cancer (*14*), recent investigations with more refined techniques do not appear to substantiate these results.

Huggins (*14*) reported an over-all reduction of acid phosphatase in the carcinomatous prostate. Downey *et al.* (*13*) gave the same report in 1954. This may be due to the inability of the rapidly dividing cells of the carcinoma to synthesize the enzyme rather than to its diffusion into the stroma, as Downey and his colleagues claimed.

Antiandrogenic measures such as castration and estrogen therapy have been widely used in treatment of carcinoma of the prostate. There has, however, been a great deal of discussion about the mechanisms of action of the estrogen on the prostatic epithelium. Szego and Roberts (*15*) claim that estrogens have to be activated by the liver before they can have any effect, and Chamorro (*16*) believes that they act indirectly by restriction of production of androgenic hormones. However, Lasnitzki (*17*) has shown in tissue culture of mouse prostate that estrogens act directly on the prostatic epithelium.

A number of investigators have suggested that estrogen phosphates should be used in the treatment of prostatic carcinoma, on the assumption that they would be dephosphorylated in the prostate by phosphatases and thus presumably more effectively localized there. It therefore appeared important to see whether one of these phosphates (such as diethylstilbestrol diphosphate) could be dephosphorylated *in vitro* by the phosphatases of the prostate.

Alkaline and acid phosphatases from prostates of humans were highly effective in hydrolyzing the diethylstilbestrol phosphate (*18*). Similar results were obtained with rat tissues, known to contain large amounts of alkaline and acid phosphatases. This ability of prostatic tissue to hydrolyze diethylstilbestrol phosphate might justify the claim of Druckrey and Raabe (*19*) that diethylstilbestrol phosphate has an organ-specific chemotherapeutic effect in the treatment of carcinoma. We found, however, that this substance was dephosphorylated by tissues other than the prostate in the rat. But there is still a possibility that the human carcinomatous prostate might be specific in this respect. It remains to be seen whether the ability of the prostate to hydrolyze diethylstilbestrol phosphate is due to its nonspecific acid phosphatases or to a specific phosphatase. Bourne (*20*) showed histochemically, at *p*H 9.0, substantial differences in the localization of phosphatases in rat tissues when diethylstilbestrol phosphate was used as a substrate, as compared with glycerophosphate or even with estrone phosphate. This suggests that there is at least one specific estrogen phosphatase. Furthermore, recent studies by Sandler and Bourne (*21*) suggest that the estrogen phosphatases may be specific and almost certainly different from glycerophosphatases.

A further problem we considered was the possibility of increasing the therapeutic value of estrogen phosphate in the treatment of prostatic carcinoma by incorporating P^{32} in the molecule (*19*). Thus it might be possible to use two therapeutic agents, the diethylstilbestrol molecule and radioactive phosphorus, to attack the malignant prostatic cells.

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PLATES

In the following figures L = lumen; S = stroma.

Plate 5

Figure 1.—Alkaline phosphatase reaction in normal mouse prostate. Positive reaction in basement membranes and periacinar connective tissue. Gomori's calcium-cobalt method. Celestine blue counterstaining. × 525

Figure 2.—Acid phosphatase reaction in normal mouse prostate. Intense positive reaction in Golgi regions (*arrows*). Nuclei faintly positive. Gomori's lead nitrate method. No counterstaining. × 450

Figure 3.—Acid phosphatase reaction in normal mouse prostate (azo-dye method). The sites of activity appear as small granules evenly distributed throughout the cytoplasm. Nuclei remain negative. Rutenburg and Seligman's postcoupling method. × 525

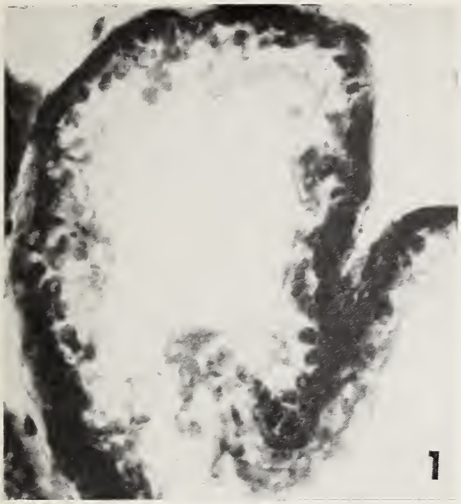
Figure 4.—Periodic acid-Schiff (PAS) reaction in normal mouse prostate. Positive material is seen at apical pole. Periacinar connective tissue shows a weak positive reaction. Celestine blue counterstain. PAS method after McManus. × 525

Figure 5.—Golgi preparation of normal mouse prostate. Golgi apparatus (*arrows*) is seen as a granular precipitate. Da Fano's silver method. × 525

Figure 6.—PAS reaction in prostate of castrated mouse. Basement membrane and periacinar connective tissue are strongly positive. × 675

Figure 7.—Alkaline phosphatase reaction in prostate of 8-day castrated mouse. The reaction at basement membrane appears discontinuous. *Arrow* points to a site where it is still positive. × 525

Figure 8.—Acid phosphatase reaction in prostate of 21-day castrated mouse. The over-all reaction is greatly decreased. Gomori's method. × 450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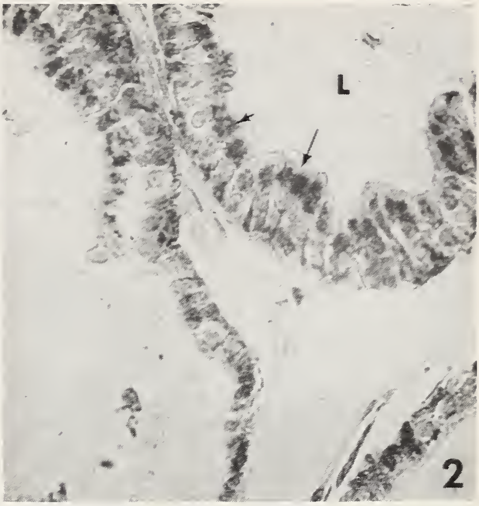
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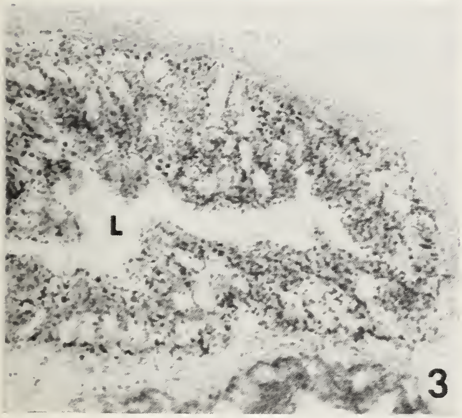
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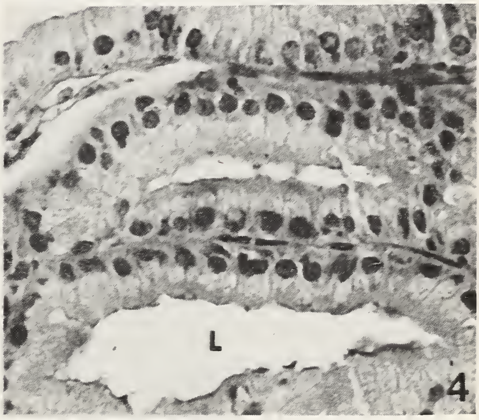
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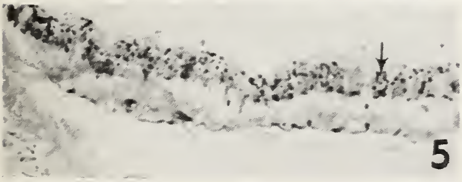
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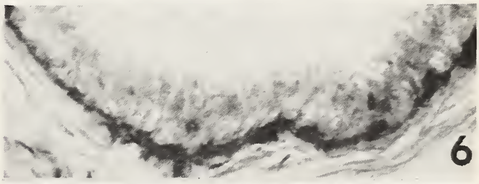
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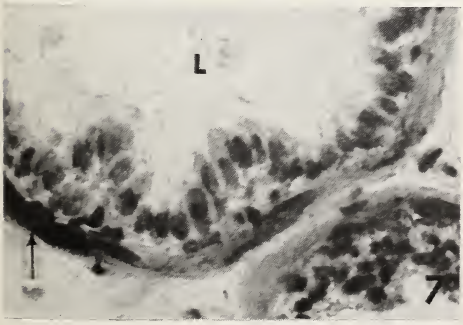
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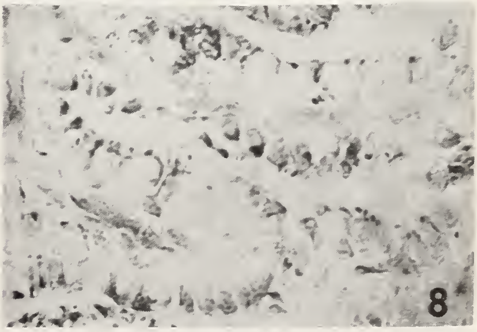
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PLATE 6

FIGURE 9.—Alkaline phosphatase reaction in prostate of mouse castrated for a longer period than that in figure 7. The reaction has completely disappeared from the basement membrane and is positive in sheaths of the hyperplastic smooth muscle stroma. × 450

FIGURE 10.—Golgi preparation of prostate of castrated mouse. Golgi apparatus has failed to become impregnated. Some debris apparently impregnated with silver are attached to the luminal border. × 525

FIGURE 11.—Alkaline phosphatase reaction in mouse castrated for 3 weeks and then treated with testosterone. Region of the basement membrane has again become positive and appears as a continuous line. Sheaths of smooth muscle fibers have retained a strong positive reaction. × 450

FIGURE 12.—Acid phosphatase reaction in mouse castrated for 3 weeks and then treated with testosterone. Positive reaction of the Golgi region (*arrows*) has reappeared. Nuclei are weakly positive. × 450

FIGURE 13.—Acid phosphatase reaction of prostatic homograft treated with 3-methylcholanthrene. Squamous cell metaplasia. Only nucleoli and what appears to be keratohyalin granules show a positive reaction. × 450

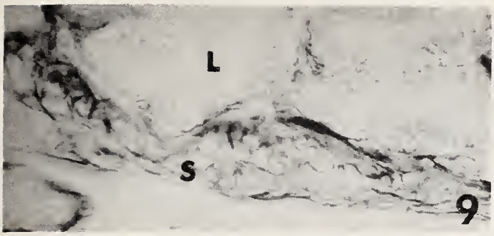
FIGURE 14.—Esterases reaction in normal human prostate, showing sites of activity in the epithelium. α-Naphthol acetate method. × 200

FIGURE 15.—Esterase reaction in human prostatic carcinoma, showing decreased activity in epithelium. α-Naphthol acetate method. × 200

FIGURE 16.—Acid phosphatase reaction in normal human prostate. Sites of activity are localized to the cytoplasm of epithelial cells, in the form of granules of moderate size. Gomori's lead nitrate method. × 525

FIGURE 17.—5-Nucleotidase reaction in human prostatic carcinoma. Activity is localized in stromal and epithelial nuclei. × 300

FIGURE 18.—Alkaline phosphatase. Human normal prostate. Reaction is positive in wall of blood vessels (*arrows*). Gomori's method. × 350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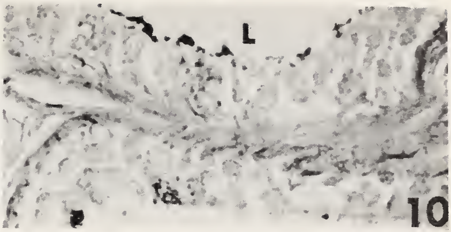
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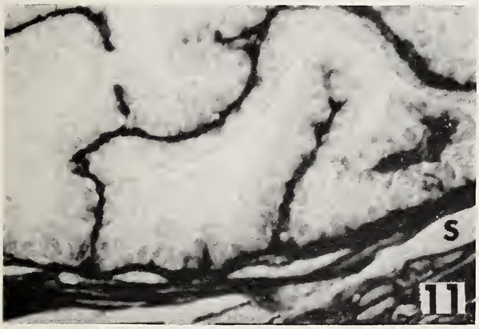
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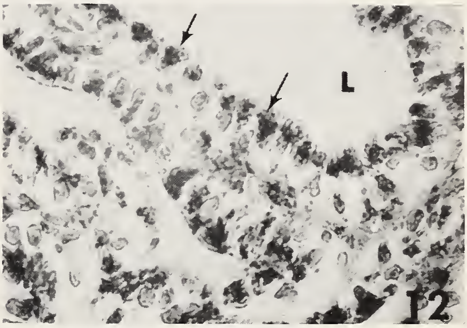
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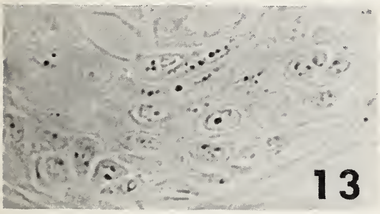
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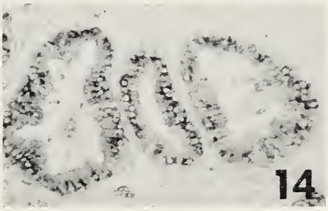
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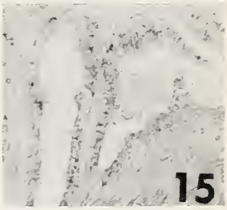
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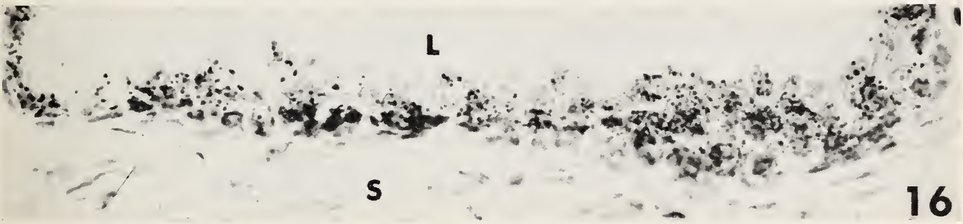
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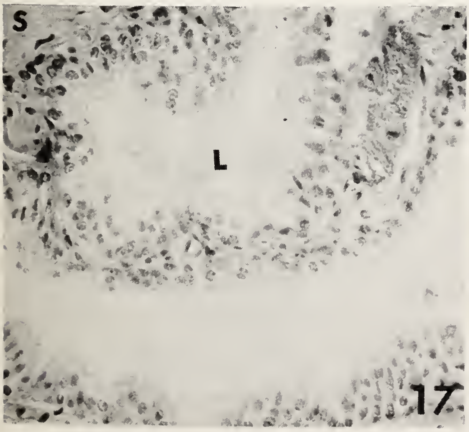
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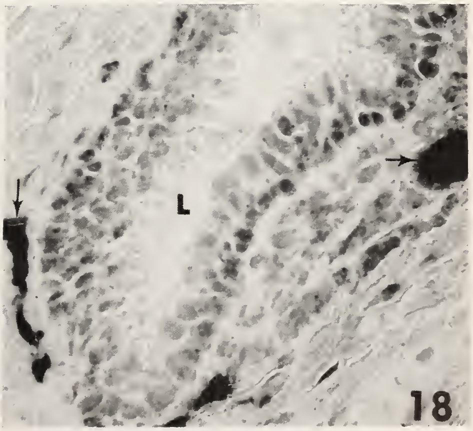
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EPITHELIAL METAPLASIA IN THE PROSTATE AND OTHER GENITAL STRUCTURES OF MALE MAMMALS[1]

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During a series of experiments concerned with the reactions of various male mammalian sex accessories to estrogen administration and vitamin A deficiency (*1–8*), several instances of epithelial metaplasia were observed in some detail. From these observations we propose to extract information on the modulative responses of the epithelia of the prostate and other accessory glands to various stimuli, and thus to establish the alternative paths of differentiation open to these epithelia.

Pioneer observations of Lacassagne (*9*) and Burrows (*10*) revealed that estrogen treatment will cause extensive squamous metaplasia and keratinization of the anterior prostate (coagulating gland) of the mouse. Earlier workers thought that the resultant overgrowths might be tumorous in nature. Less dramatic metaplastic alterations can also be seen in other regions of the prostatic complex and in the seminal vesicle (*4*). These alterations begin as proliferations of basal cells beneath the original epithelium, which may eventually replace the latter. Proliferating islands of cells can be recognized by their distinctive content of alkaline phosphatase (*4, 5*) and by other cytochemical characteristics (*7*), which point to intense synthetic activity.

Distinct site and species differences exist in the response to estrogen (*5, 6*). Four common laboratory mammals may be arranged according to extent of the metaplastic response of their male sex accessories to estrogen as follows: mouse > guinea pig > rabbit > rat. However, the nature of the response also must be considered. In the distal regions of the seminal vesicle and in some lobes of the prostate in the mouse, true epidermoid metaplasia is not encountered after estrogen (*4, 5*); instead the advanced picture is one of noninvasive basal cell accumulations. The ventral prostate does not show metaplasia (*4*). In the guinea pig, signs of cornification are relatively infrequent (*5, 6*). In the rabbit, keratinization is never encountered; the metaplastic response to estrogen is "transitionoid" or parakeratotic in nature (*1–5*). It appears that the genetic determinants of genital epithelial response *to estrogen* in both male and female rabbits do not permit cornification.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

It has long been known that avitaminosis A will result in keratinizing lesions in genital epithelia as in other epithelia (*11*). The keratinizing metaplasia of vitamin A deficiency in the mouse prostate resembles, in its ultimate histologic appearance, metaplasia resulting from estrogen treatment. However, the alkaline phosphatase activity associated with estrogen treatment is absent from the avitaminotic A lesions (*5*). The genital epithelial metaplasia after deprivation of vitamin A is much more extensive in the mouse than that seen after estrogen treatment; however, the anterior prostate remains the most sensitive area. Lesions are found in the epididymis and the ductus deferens, as well as in structures located adjacent to the urethra. The sex accessories of the rat, which show only a limited metaplastic response to estrogen, respond with widespread squamous metaplasia to deprivation of vitamin A. As in the mouse, the metaplastic epithelium is alkaline phosphatase-negative (*5*).

The particular noncornifying response of the rabbit to estrogen is of special interest, because the avitaminotic A syndrome is apparently the same in this mammal as in others that have been investigated experimentally (*11*). Furthermore, spontaneous keratinizing lesions of unknown etiology have been observed commonly in the prostate and the vesicular gland of otherwise normal rabbits (*3*). These lesions are highly reactive for alkaline phosphatase; this suggests that they may have no relation to vitamin A deficiency. The administration of large amounts of vitamin A does not prevent their occurrence (*3*). Their existence establishes the ability of the prostatic epithelium in the rabbit to undergo both parakeratotic ("transitionoid"?) metaplasia and keratinizing metaplasia.

Distinctions between epithelial metaplasia in the genital tract consequent to avitaminosis A and that consequent to estrogen administration have been outlined earlier (*5*). An explanation on embryologic grounds of the occurrence of estrogen-induced epidermoid metaplasia in the male genital tract has been offered by Zuckerman (*12*), to the effect that such estrogen-responsive epithelia are of urogenital sinus origin. The possibility of an ectodermal contribution to the urogenital sinus, which might account for the epidermoid nature of the metaplastic response, has also been raised. Recently, Raynaud (*13*) strongly reaffirmed Zuckerman's hypothesis, and some Russian workers also favor a "germ-layer" explanation of epithelial metaplasis in other sites [*see*, for example, the Discussion of "metorhizis" in (*14*)]. It is not the purpose of this comment to reopen debate on this point. However, the occurrence of keratinizing squamous metaplasia, following vitamin A deficiency, in genital structures having no relation to the urogenital sinus suggests that the ability to keratinize under some circumstances may be a fundamental property of the basal cells of all epithelia in mammals, regardless of germ-layer origin (*cf 15*).

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FUNCTIONAL ULTRASTRUCTURE OF RAT PROSTATIC EPITHELIUM[1, 2]

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It has been postulated that the ribonucleoprotein particles (RNP) attached to the limiting membranes of the ergastoplasm and those free within the cytoplasmic matrix are responsible for cytoplasmic basophilia (*1–5*). Circumstantial evidence supports the assumption that the ergastoplasm and associated RNP particles are involved in protein synthesis (*2*, *6–9*). It has also been shown that the RNP particles manifest a high and rapid uptake of radioactive amino acids (*10*), which indicates their active role in protein synthesis (*5*).

In previous communications (*11*, *12*) it was demonstrated that the epithelial cells of the rat ventral prostate possess a highly developed ergastoplasm. The rate of incorporation of glycine-2-C^{14} into the prostate proteins was decreased after the administration of estrogen. This hormone also produced a collapse and depletion of the ergastoplasmic sacs (*13*). A fine structural analysis of the effect of castration upon the rat ventral prostate is presented here. We have also tried to correlate the ultrastructural appearance of the prostatic epithelial cells with functional and biochemical changes known to occur during androgen deprivation (*14–16*).

MATERIALS AND METHODS

Holtzman albino rats weighing 250 to 350 g were used. Castration was performed under ether anesthesia, through a double scrotal incision. We made observations 2, 4, 8, 12, 15, 19, 25, and 30 days after castration, using 4 animals for each time point. Controls were intact animals of approximately the same weight, some of which had undergone sham operations. All the animals maintained or gained weight from the date of castration to the time of observation.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] This investigation was supported by research grants C-5425 (Path.) National Cancer Institute, and H-4178 (Path.), National Heart Institute, National Institutes of Health, Public Health Service.

The removal of specimens for electron microscopy was performed under ether anesthesia. The ventral lobes of the prostate glands were cut into small fragments which were then fixed for 1 hour at 4° C in Caulfield's (*17*) osmium tetroxide fixative. The tissues were then washed briefly in distilled water, dehydrated in increasing concentrations of ethyl alcohol, and embedded in a mixture of 1 part of methylmethacrylate and 19 parts of *n*-butylmethacrylate containing 2 percent benzoyl peroxide as a catalyst. Polymerization was obtained by exposure to a temperature of 56° C for 48 hours. Sections were cut with an Ultrotome (LKB) and some were stained with uranyl acetate (*18*). Electron micrographs were taken with an RCA electron microscope model EMU-3F at original magnifications of 3,000 to 17,500 and enlarged to the desired size.

Acid phosphatase was demonstrated by the method of Rutenburg and Seligman (*19*) in unfixed sections cut with the cryostat. Basophilia was demonstrated by the gallocyanin method of Boer and Sandaker (*20*).

Protein synthesis was measured by the rate of glycine-2-C^{14} incorporation into protein. The exact experimental details have been described elsewhere (*13*). Normal male rats were compared with rats castrated 2, 4, 7, and 16 days before each experiment. Castrated rats receiving either testosterone (Oreton, Schering) or nandrolone phenpropionate (Durabolin, Organon) were injected intramuscularly with 0.5 mg of either hormone. Hormone-dependent protein synthetic capacity was then measured with glycine-2-C^{14} at 24, 48, 72, and 96 hours after injection of the hormone.

RESULTS

Untreated Rats

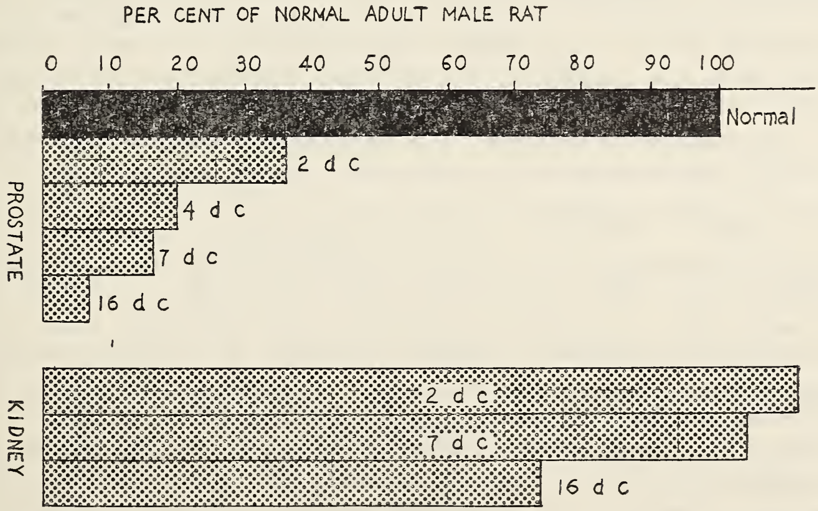
In light micrographs of gallocyanin preparations (fig. 1) the prostatic acini appear lined by tall columnar epithelium. The nuclei are located in the basal portion and are intensely stained. According to the distribution of basophilia, the cytoplasm can be divided into four regions: the apical (A), Golgi (G), supranuclear (S), and basal (B). With the exception of the Golgi which appears as a clear space, the other regions show marked basophilia.

In electron micrographs the tall columnar epithelial cells (fig. 2) appear surrounded by the plasma membrane, and the cytoplasm shows two phases: a moderately dense phase consisting of the cytoplasmic matrix and most of the cell organelles, and a second phase of lesser density representing dilated ergastoplasmic sacs. The distribution of the ergastoplasm resembles that of the basophilic substance. It is abundant in the apical (A), supranuclear (S), and basal (B) regions, but it is very scarce in the Golgi (G) zone. On higher magnification (fig. 3) the cytoplasmic matrix shows amorphous substance and numerous RNP granules, some of which are arranged in clusters. The mitochondria reveal the usual structural arrangement; they are surrounded by a double membrane and show numerous cristae. The ergastoplasm appears as a system of highly dilated sacs containing low opacity material. These sacs are separated from the cytoplasmic matrix by limiting membranes with RNP granules attached to their outer aspect (fig. 3).

Castrated Rats

The most striking change observed in electron micrographs of prostatic epithelium of castrated rats is a gradual collapse of the dilated ergastoplasmic sacs and the depletion of their amorphous content (figs. 4 and 5). With the suppression of androgenic stimulation for 8 days or more, the ergastoplasmic system of intercommunicating channels, which formerly occupied such a large proportion of the cytoplasm, is now represented only by a few isolated flattened or oval profiles (fig. 6). There is also a progressive decrease in the number of both membrane-bound and free ribosomes (fig. 6). At this period there is a marked decrease of cytoplasmic basophilia in gallocyanin preparations (fig. 7).

While these changes occur, large numbers of dense bodies appear in the cytoplasm of the epithelial cells (figs. 4, 5, 6, and 9). These dense bodies, showing highly polymorphic inner configuration, may be isolated (fig. 4), in groups (fig. 5), or as conglomerations of dense bodies and cell debris (figs. 6 and 9). When isolated or in groups, the dense bodies tend to be oval and are usually bounded by single membranes. Their matrices may be homogeneous (fig. 6), of moderate to marked opacity, or of granular texture (figs. 4, 5, 6, and 9). Some contain one or more oval



Text-figure 1.—A bar graph comparison of the rate of glycine-C^{14} incorporation into ventral prostate and kidney protein of normal adult rats and rats castrated 2, 4, 7, or 16 days previously. Each bar represents the numerical average of 6 to 12 separate determinations during a period in which specific activity of the proteins increased in essentially linear fashion with respect to time—d = days, c = castrated.

vacuoles containing material of low opacity (figs. 4 and 9). Staining for acid phosphatase (fig. 8) shows a positive reaction in the form of coarse granules corresponding in size and location with the dense bodies seen in the electron micrographs (figs. 4, 5, 6, and 9). When the castration period is prolonged (12 days or more) the involution of ergastoplasm and the decrease of cytoplasmic ribosomes become prominent (fig. 9).

The biochemical studies showed a dramatic effect of castration on the ventral prostate when the rate of glycine-2-C^{14} incorporation into protein was measured at various time points. Castration caused such rapid loss of protein synthetic capacity that, after 2 days, less than 40 percent of that capacity remained (text-fig. 1). At 4 and 7 days this effect intensified. Rats castrated up to 16 days beforehand demonstrated greater loss of protein synthetic capacity, which corresponded in the electron micrographs with a marked involution of the ergastoplasm and a decrease in the number of cytoplasmic ribosomes.

From the kidney correlative information was obtained. Up to 7 days following castration, no significant change was evident in protein synthetic capacity. However, 16 days of hormone deprivation caused significant diminution in the rate of glycine-2-C^{14} incorporation into protein.

DISCUSSION

The present observations indicate that the characteristic topographic distribution of basophilia in the prostatic epithelial cells of normal rats (*21*, *22*) corresponds, in the electron micrographs, to the well-developed ergastoplasmic sacs and attached ribosomes and to the presence of free ribosomes in the cytoplasm. No basophilia, however, can be detected in the Golgi region where the electron micrographs reveal an almost complete absence of ribosomes. The membranes in this zone are mainly agranular and therefore of the Golgi type. Hence, it seems that in rat prostatic cells, cytoplasmic basophilia occurs in sites characterized by the presence of ribosomes, either free or attached to membranes. In this particular instance, such sites rich in ribosomes also present a highly developed ergastoplasmic component, and the possible contribution of this component to basophilia must be considered. For other cell types the degree of cytoplasmic basophilia observed in histologic sections apparently is correlated with the presence of ribosomes rather than with the extent of development of cytoplasmic membranes (*1–5*, *23*, *24*).

These experiments confirm endocrine control of the fine structural organization of prostatic epithelial cells (*11*, *25*) in the particular and profound alteration of the ergastoplasm-ribosome complex by androgen deprivation. Strong circumstantial evidence deeply implicates this complex in the synthesis and storage of cellular protein products (*2–6*, *10*, *26–30*). Involution of the complex and loss of cytoplasmic basophilia as shown here coincide with suppression of secretory activity after castration (*31*). These evidences of synthetic activity reappear on the application of androgen (*32*). It may then be fairly assumed that in prostatic epithelial cells the ergastoplasm-ribosome complex is at least partly responsible for the production of secretory material and that its full development is signaled in light micrograph preparations by cytoplasmic basophilia. These morphologic deductions are supported by the dynamic demonstration of sharply reduced glycine-2-C^{14} uptake in protein of rat prostate coinciding with estrogen (*13*) or castration-induced involution described.

The numerous dense bodies in the prostatic epithelium of castrated rats have the fine structural characteristic and acid phosphatase activity of DeDuve's (*33*) lysosomes. In establishing their functional significance, we must first consider the marked involutionary changes occurring in these cells following castration, as shown by their atrophy. Furthermore, the collapse of the ergastoplasmic sacs suggests the removal of large masses of secretory proteins, while the almost complete disappearance of ribosomes and cytoplasmic basophilia indicate a marked decrease of ribonucleic acid content. It therefore can be postulated that the development of large numbers of lysosomes in the prostatic cells of castrated rats is related to the involution of the epithelium and the degradation and removal of cellular material by the hydrolytic enzymes contained in the lysosomes (*34*).

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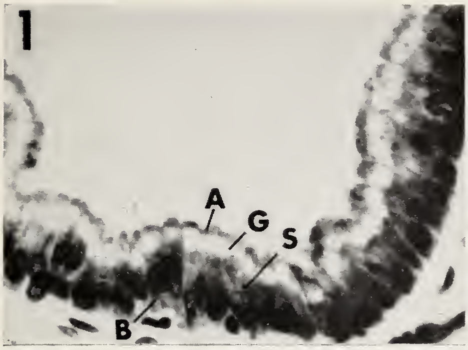
PLATES

Plate 7

Figure 1.—Light micrograph of ventral prostate in normal rat. Apical (A), supranuclear (S), and basal (B) regions appear basophilic. The Golgi (G) remains unstained. Nuclei also show intense basophilia. Gallocyanin stain. × 250

Figure 2.—Electron micrograph of ventral prostate in normal rat. Survey picture. Columnar epithelial cells are bound by plasma membrane (Pm). Well-developed ergastoplasm is visible in apical (A), supranuclear (S), and basal (B) regions, but not in the Golgi (G). *Note* correspondence between the presence of ergastoplasm in electron micrograph and distribution of basophilia seen in figure 1. × 4,600

Figure 3.—Electron micrograph of ventral prostate in normal rat. On higher magnification, the dilated ergastoplasmic sacs (Er) show a homogeneous content of low opacity. Numerous ribosomes (Rb) are attached to limiting membranes of the ergastoplasm and also free in cytoplasmic matrix. × 40,000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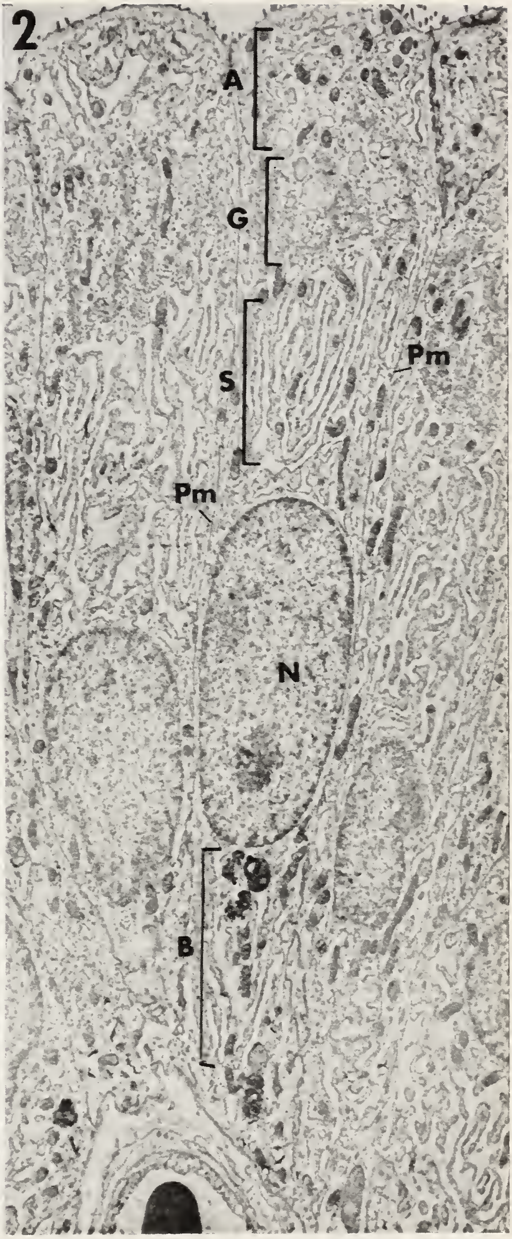
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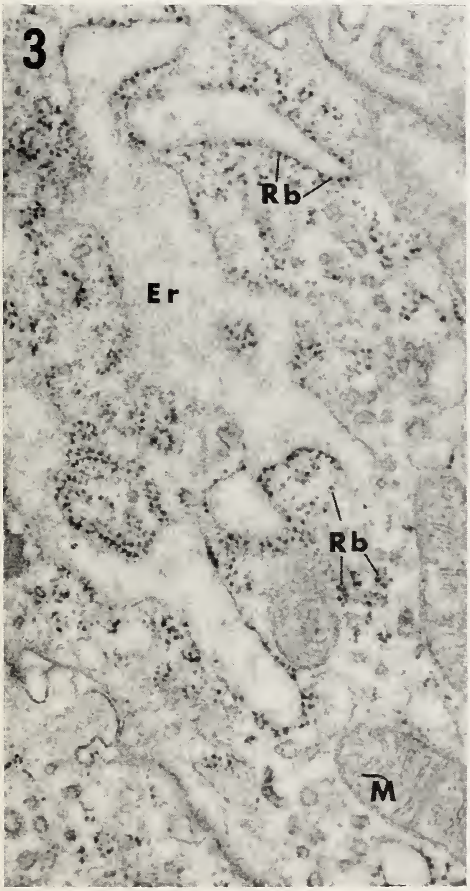
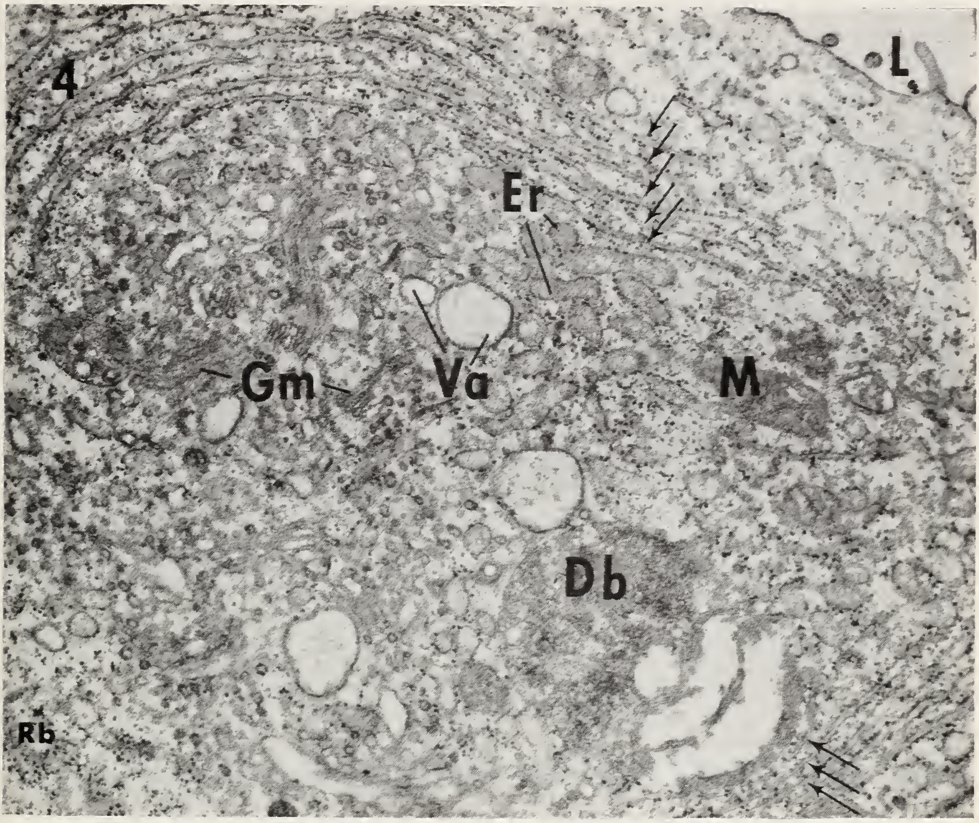

3
Rb
Er
Rb
M

PLATE 8

FIGURE 4.—Electron micrograph of ventral prostate in a 4-day castrated rat. It shows flattened ergastoplasmic cisternae (*arrows*) arranged concentrically around an area containing Golgi membranes (Gm), Golgi vacuoles (Va), mitochondria (M), and vesicular profiles (Er) of the ergastoplasm. A dense body (Db) showing granular texture and internal vacuoles is also visible. Ergastoplasmic sacs are studded with ribosomes, also present in cytoplasmic matrix (Rb). × 28,000

FIGURE 5.—Electron micrograph of ventral prostate in a 4-day castrated rat. Basal region. *Arrows* indicate collapsed ergastoplasmic sacs. A few sacs (Er) remain dilated. Membrane-bound and free ribosomes (Rb) are still abundant. Two dense bodies (Db) show areas of variable density. Mitochondria (M) and part of nucleus (N) are also visible. Basement membrane (Bm) appears as a layer of homogeneous material attached to outer aspect of the plasma membrane (Pm) × 28,000

4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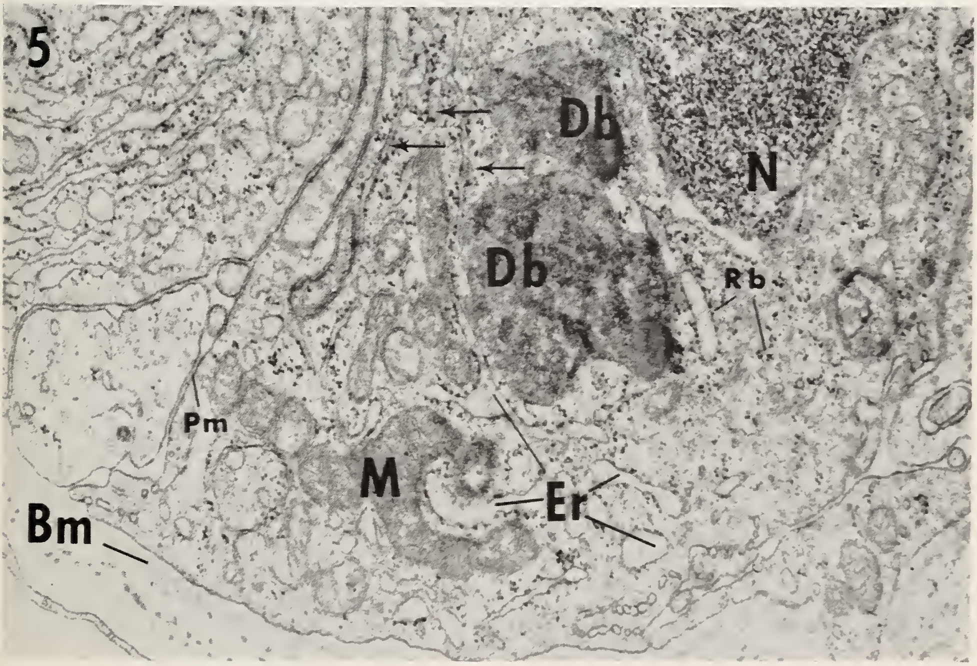
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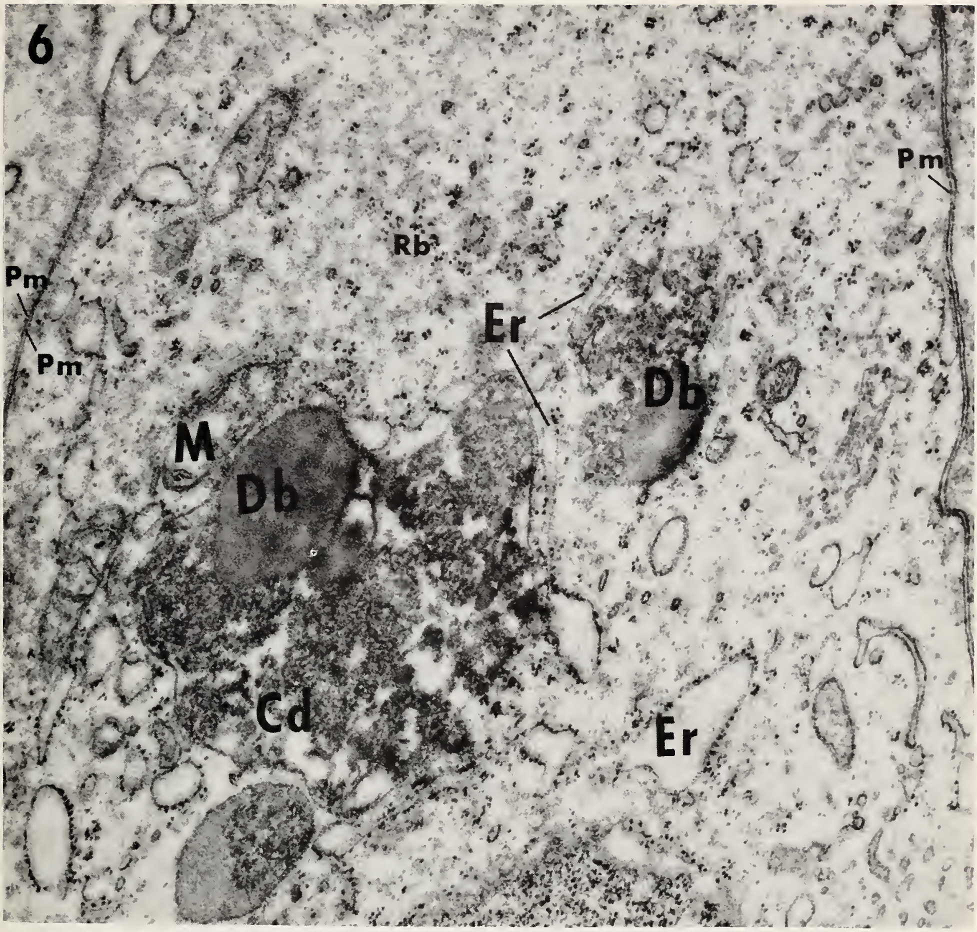
PLATE 9

FIGURE 6.—Electron micrograph of ventral prostate in 8-day castrated rat. Ergastoplasm (Er) has undergone further involution, vesicular and flattened elements being separated by wide areas of cytoplasmic matrix. Conglomerations of dense bodies (Db) are associated with cellular debris (Cd). Plasma membranes of two adjacent cells (Pm) are also shown. Free and membrane-bound ribosomes (Rb) are still plentiful. × 37,500

FIGURE 7.—Light micrograph of ventral prostate in 8-day castrated rat. Cytoplasm shows little basophilia, but nuclei still stain intensely. *Compare* with the zonal distribution of basophilia in the normal (fig. 1). Gallocyanin stain. × 250

FIGURE 8.—Light micrograph of ventral prostate in 8-day castrated rat. Large acid phosphatase-positive conglomerates (*arrows*) can be seen in the apical and basal regions. These enzyme-rich conglomerates seem to correspond with dense bodies seen in similar locations in electron micrographs in figures 4, 5, and 6. Nuclei (N) appear negative. × 525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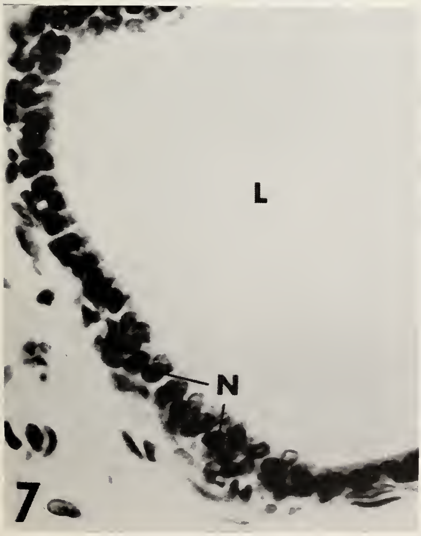
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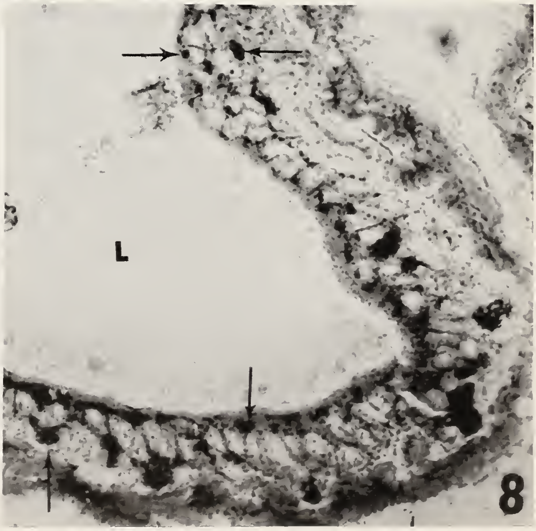
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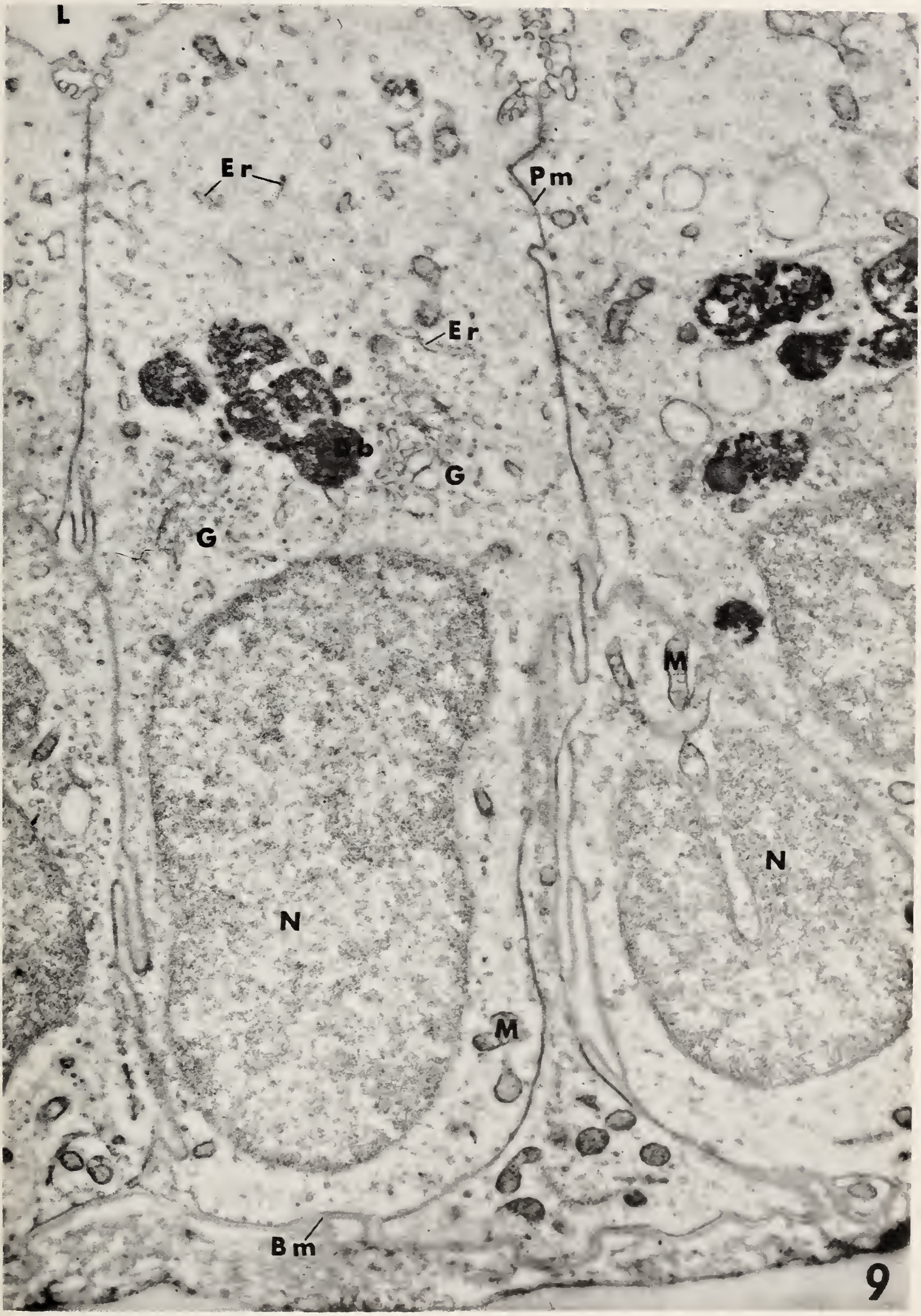
PLATE 10

FIGURE 9.—Electron micrograph of ventral prostate of 19-day castrated rat. *Note* the almost complete disappearance of free ribosomes. Remnant of ergastoplasm is represented by a few isolated shrunken profiles (Er). Conglomerates of dense bodies (Db) can be seen in neighborhood of the Golgi apparatus. Basement membrane (Bm) seems unchanged. *Note* correlation between the marked involution of ergastoplasm-ribosome system and the great inhibition of protein synthetic activity observed at this period after castration (text-fig. 1). × 20,000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DISCUSSION

Dr. Williams-Ashman: I would like to ask Dr. Brandes one question. If you examine the tissues in the ventral prostates of the mouse and the rat by electron microscopy at various intervals after castration, can you see any change in the ribosomal population density either before or after any change in the distention of the ergastoplasmic sacs?

Dr. Brandes: In the first few days after castration, especially after 24 or 48 hours, the ergastoplasm seems to dilate and widen, and the changes in the ribosomal population seem difficult to estimate. It is not possible to say. It is only at later periods that we can forecast.

Dr. Williams-Ashman: How many days after castration will you have a definite decrease in the ribosomal population?

Dr. Brandes: About 8 or 10.

Dr. Deane: What method was used for the acid phosphatase when you got nuclear stain?

Dr. Bourne: The Gomori procedure.

Dr. Deane: I think it is wise to mention here that the procedure—depending on precipitation, ultimately, of lead sulfide at the site of enzymatic activity—may result in false localization. It is not so much that the total amount of enzymatic activity is falsely represented, but that the location of that activity may be false. It is important to perform elaborate control preparations to satisfy oneself as to location. Nuclear staining in particular is problematical. Usually what appears to be nuclear localization occurs in situations in which the formation of the product is slow and, therefore, there is time for migration of the product to the nuclei.

Dr. Bourne: That is true. However, if this is an artifact it is one altered by castration and reproduced in its original form by testosterone. So whether the actual concentration of the enzyme is in the nuclei is not significant. The results indicate that castration has done something to the cell which has affected the distribution of the enzyme.

Dr. Schirmer: To Dr. Bourne's findings on the presence of acid phosphatase in the Golgi apparatus I would like to add evidence from another method—that of fractionation of cells in the prostate of the dog and ultracentrifugation. I have found that by this method 10 percent of the acid phosphatase as analyzed with Bodansky's method was localized in the mitochondria, 70 percent in the microsomal fraction, and 20 percent in the supernatant. This might support your findings.

ELECTRON MICROSCOPIC OBSERVATIONS ON THE MOUSE SEMINAL VESICLE[1, 2]

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THIS paper presents some observations on the fine structure of the epithelium of the seminal vesicle in the normal adult mouse. To date there have been relatively few reports on the electron microscopy of this tissue (*1–4*), and my own experience suggests that the difficulty of fixing it has limited such study. To be specific, preparative methods that prove satisfactory for a wide variety of other cell types result in the apparent distention of the ergastoplasmic channels of the secretory cell in this epithelium (*3*). Preparations used in this report were fixed in an osmium tetroxide solution containing 10 percent (0.3 M) sucrose, which prevented gross distention of the ergastoplasmic channels (*5*). In addition, results with fixatives containing other concentrations of sucrose or sodium chloride will be indicated.

MATERIAL AND METHODS

Observations were made on the glands of 13 young adult mice, some of them Swiss albinos provided by a local dealer, some hybrids contributed by Dr. Lois Jean Smith and Mrs. Anna Chao Pai.

The fixing solution adopted after considerable experimentation was 2 percent OsO_4 in 0.1 M phosphate buffer of *p*H 7.8 (final *p*H of mixture, *ca.* 7.4), containing 10 percent (0.3 M) sucrose (*5*). This concentration of sucrose empirically prevented the otherwise excessive distention of the ergastoplasmic cisternae. Increased concentration of OsO_4 was tried because of the abundance of membranes in the tissue. Phosphate buffer was adopted because of its great stability near neutrality; moreover, Millonig (*6, 7*) has shown that it allows preservation of more constituents of protoplasm than does the Veronal-acetate buffer in the Palade mixture used in our first study (*2, 3*). The material was embedded in an epoxy

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] This research was supported by grant E-205 from the American Cancer Society, Inc., and grant B-2145 from the National Institutes of Health, Public Health Service.

[3] Valuable technical assistance was given by Miss Arline Albala and Mr. Stanley Brown.

resin (*8*), which produces sections far superior to those from blocks embedded in methacrylate.

Small pieces of gland were immersed in a drop of the chilled fixative, diced with a razor blade, transferred to a large volume of the same fixing solution, and left for 2½ to 3 hours at 4° C. The blocks were then washed in chilled buffer and trimmed to smaller size, dehydrated with increasing concentrations of chilled ethanol, brought to room temperature in absolute ethanol, cleared with propylene oxide, and infiltrated with the epoxy resin mixture. The latter approximated Luft's (*8*) 5:5 *A*:*B* combination: 5 parts of Shell Epon 812,[4] 3 parts of the hardener DDSA,[5] and 2 parts of the hardener NMA,[5] plus 1.5 percent of the accelerator DMP-30.[6]

The encapsulated blocks were cured for 24 hours each at 25, 35, 45, and 60° C, after which they were hard enough to be cut on a Porter-Blum microtome with a Dupont diamond knife at approximately 80 mμ (silver-to-gold).

The ribbons were mounted on copper grids, 200 mesh per inch, that had been coated with a thin layer of collodion made from a 0.2 percent solution in amyl acetate. On the same day, the mounted sections were "stained" for 2 hours in a 0.5 percent solution of uranyl acetate in 50 percent ethanol at room temperature. Afterward they were coated with a thin layer of carbon. Micrographs were taken in an RCA EMU-3E, operated at 100 kv, on Ilford N.60 photographic plates at initial magnifications of 1,800 to 10,600 and enlarged twofold to fourfold.

In addition to the standard fixative given, others tested contained no additive, or sucrose at different concentrations between 5 and 20 percent (0.15–0.6 M), or sodium chloride at concentrations ranging from 0.5 to 3 percent (0.15–1.0 osmolar).

OBSERVATIONS

Normal Cytology

The seminal vesicle epithelium of a young adult mouse is shown in figure 1. The tissue is composed principally of tall, columnar cells; interspersed among their bases are the so-called basal cells, which are stellate, exhibiting many cell processes. The cells rest on a delicate basement membrane, which overlies a connective tissue space sparsely populated with collagen fibrils and containing small blood vessels and a few connective tissue cells.

Adjacent epithelial cells are locked together by occasional desmosomes and some degree of interdigitation, particularly near their bases (figs. 1, 2, and 6).

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[4] The epoxy was generously donated by Miller Stephenson Chemical Co., Inc., Danbury, Conn.

[5] Dodecenyl succinic anhydride and nadic methyl anhydride were kindly provided by Allied Chemical Corp., Plastics Division, New York, N.Y.

[6] 2,4,6-Tri(dimethylaminoethyl)phenol was kindly donated by Rohm and Haas Co., Philadelphia, Penna.

The secretory cells have a single somewhat ovoid nucleus, which contains a nucleolus, conspicuous masses of chromatin, and many pores in the double nuclear membrane (figs. 1, 2, 4, and 5). The nuclear pores are roughly 1000 A in diameter. These cells are notable for their content of ergastoplasm, which occupies most of the cytoplasmic space, except for that taken by the sparsely distributed mitochondria and the large, supranuclear Golgi complex. The ergastoplasmic system consists of closely packed channels bounded by delicate membranes, to which are attached numerous ribosomes (figs. 1, 2, and 3). There is virtually no density to the contents of the channels. These are disposed in a great whorl in the cells, reaching almost to the plasmalemma. However, at the base of the cell and along the sides to about the nuclear level, there seems to be a thin subplasmalemmal layer free of ergastoplasm. In this layer are occasional coiled microtubules or rows of vesicles, which might permit at least transient continuity between the outside of the cell and the ergastoplasmic channels (figs. 3, 4, and 6).

The ergastoplasm invests the Golgi complex closely, and in occasional specimens its channels appear to make continuity with the small tubules or flattened cisternae that surround and feed into the larger secretory vesicles. This possible continuity is not evident, however, in figure 5. The ergastoplasmic channels probably conduct to the Golgi channels both proteinaceous material, which is packaged there into dense secretory granules, and the fluid in which these granules seem to float. I have no idea just how the granules are condensed and yet surrounded by fluid. Apparently the granules are released into the lumen with this fluid, by the mechanism of fusion of the Golgi membrane with the plasmalemma, followed by rupture of a point on the outer plasmalemma, as demonstrated by Palade for the pancreatic acinar cell (*9*). The cells also generally show an irregular population of microvilli on the luminal aspect (figs. 1 and 5).

Figure 6 depicts portions of at least four basal cells, as seen when the tissue is cut tangentially to the basement membrane. These are quite different from the secretory cells. They are pleomorphic. Generally, the nucelus is free from pores. The cells contain only traces of ergastoplasm or smooth-surfaced endoplasmic reticulum. The Golgi apparatus does not show any dense contents. The mitochondria are smaller and denser than in the secretory cells, relatively more crowded, and just about as complicated in their internal structure. Whether basal cells are only supportive or may, on demand, differentiate into secretory cells is not now clear.

Influence of Molarity of Fixative

Material fixed in buffered osmium tetroxide without the addition of sucrose exhibits what I interpret to be distention of the ergastoplasmic channels (fig. 7). This distention is especially evident deep in the block, which suggests that slow penetration of the fixative has permitted osmotic uptake of water. With the addition of increasing concentrations of sucrose to the fixative, the channels of the ergastoplasm are progressively smaller, so that when the additive is at a level of 15 or 20 percent (0.45–0.6 M), the channels are extremely narrow (fig. 8). It may be that my choice of 10 percent sucrose for the standard fixative was incorrect and will require further substantiation. Even with high concentrations of sucrose, the Golgi vesicles generally remain nearly as large as in specimens fixed without additive or with lower concentrations of it.

In contrast to these results with sucrose, which represent the expected response to high molarity of the fixative, those with sodium chloride were quite unexpected. At no concentration tested, between 0.5 and 3.0 percent (0.15–1.0 osmolar), did sodium chloride detectably shrink the ergastoplasmic channels (fig. 9).

DISCUSSION

The secretory cells of the seminal vesicle of the mouse [and rat (*4*)] have the cytological characteristics of cells specialized to synthesize, package, and release proteinaceous material actively. In this respect they resemble superficially those of the rodent ventral prostate, which have been studied with the electron microscope by Harkin (*10*, *11*) and Brandes and co-workers (*12*, *13*). In the ventral prostate of mouse and rat, however, the secretory material is not as dense as in the seminal vesicle. Electron microscopy has emphasized that the different glands in the male reproductive tract have little in common cytologically or in the nature of their products (*13*).

Studies on the mouse by Fujita (*1*) and Deane and Porter (*2*, *3*) and on the rat by Szirmai and van der Linde (*4*) showed that in the seminal vesicle cytological signs of secretory activity appear depressed after castration and enhanced after testosterone administration. These studies were made on material that was not as well preserved as is now possible, and no doubt many additional details would be revealed by re-examination of glands from experimental animals.

Although the data are still incomplete, I should like to consider the significance of the influence of additives to the fixative on the appearance of the secretory cell in the mouse seminal vesicle. Following are speculations that lead to working hypotheses for future substantiation:

(*a*) Fixation in buffered 2 percent osmium tetroxide without additive appears to distend the ergastoplasmic channels in seminal vesicle cells. Proof for this conclusion is incomplete, but aside from the point just mentioned, these cells show more frequent distention of such channels when fixed in this way than do many other cell types—notably various epithelial cells in the intestine or plasmacytes, several of which have abundant ergastoplasm (*5*). It is my working hypothesis that this distention of the channels in seminal vesicle cells is a response to the osmotic activity of low-molecular-weight substances in the cisternae. Such substances might include fructose or a precursor, or some of the other relatively small organic molecules contributed to the seminal fluid by this epithelium (*14*, *15*). Thus, with any standardized fixation, the relative degree of distention of the cisternae might indicate the local concentration of such osmotically active substances. For example, Porter and I reported that the cisternae appeared less distended than in control animals after castration and more distended after testosterone administration (*2*, *3*).

(*b*) If this hypothesis is correct, the results with the addition of adequate amounts of sucrose to the fixative indicate that this sugar does not penetrate the cells and can prevent the uptake of water by the cytoplasm and, secondarily, by the ergastoplasm. Alternatively, increasing concentrations of sucrose may progressively withdraw water from the cells and, hence, the channels. It is difficult to determine that either alternative is correct. Magnifications available in electron microscopy make possible observations for which there are no auxiliary checking methods. For example, it is not enough simply to determine the proper tonicity or osmolarity of a culture medium that would prevent alteration in size of these cells when living. Osmium tetroxide, like some other fixing agents, causes swelling that must be offset (*16*, *17*). Speculations in this area must be tempered by considerations of the significance of microtubules and pinocytotic vesicles, as well as of the import of the progressive subdivision of cytoplasm by internal membranes.

(*c*) Finally, the results with added sodium chloride suggest that the seminal vesicle cells are not impermeable to sodium ions, so that this substance fails to exert any osmotic pressure. Thus, their ergastoplasm does not shrink when the tissue is fixed with high concentrations of sodium chloride, even when red blood cells in the vicinity have become crenated. As a point of departure to explain this discrepancy between the seminal vesicle cell and the red cell, I suggest that since this cell transports sodium to the seminal fluid (*15*, *18*), it may be readily permeable to sodium ions and not able to reject their entry by a sodium pump of the type found in red blood cells and nerve fibers.

I offer these speculations in the full knowledge that they are as yet unconfirmed. Perhaps their chief attribute is the indication that even experiments designed to improve quality of fixation of a tissue may allow a certain amount of cytophysiological analysis. The next task is to devise other techniques to test the speculations.

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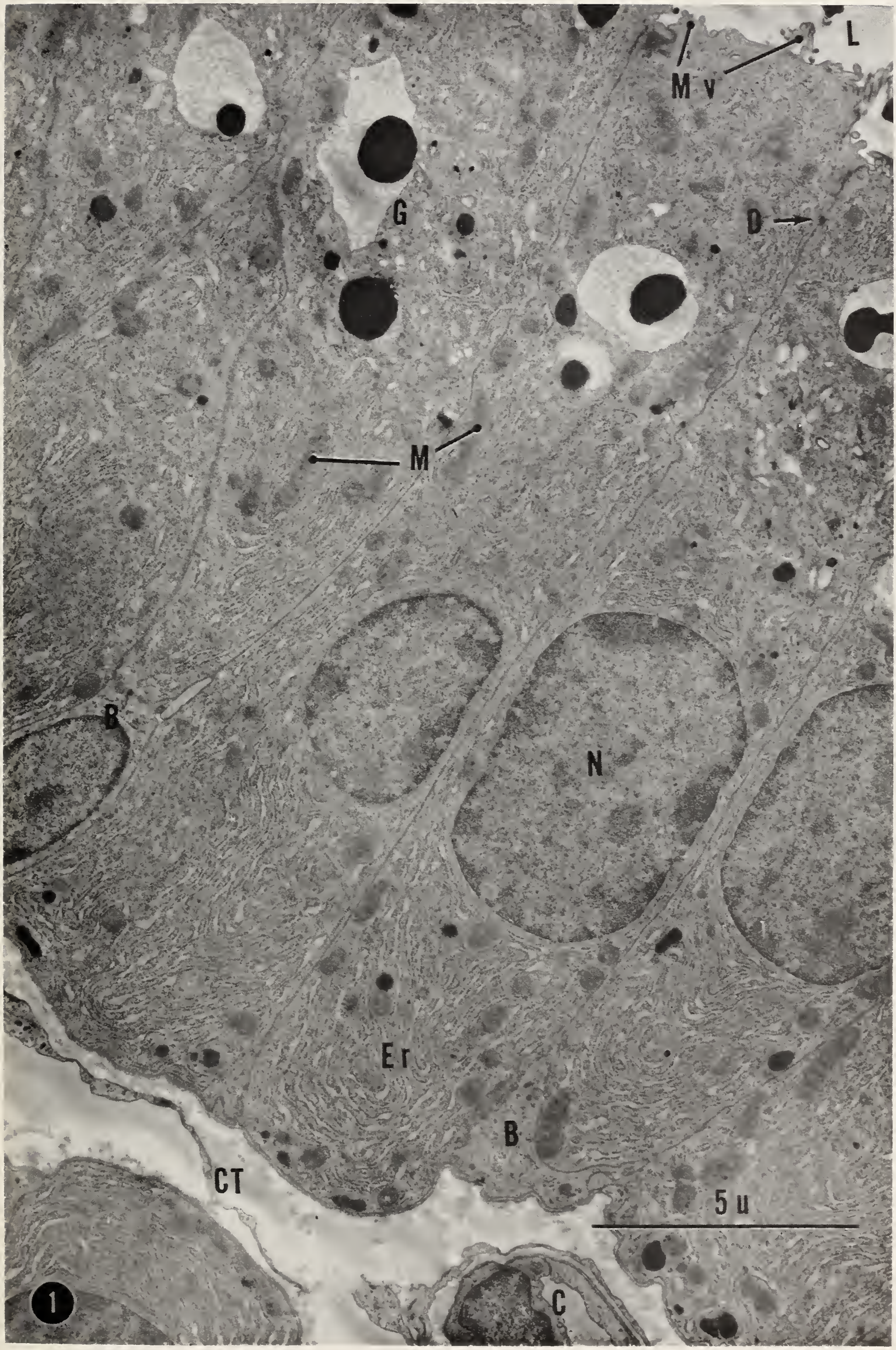
PLATES

Plate 11

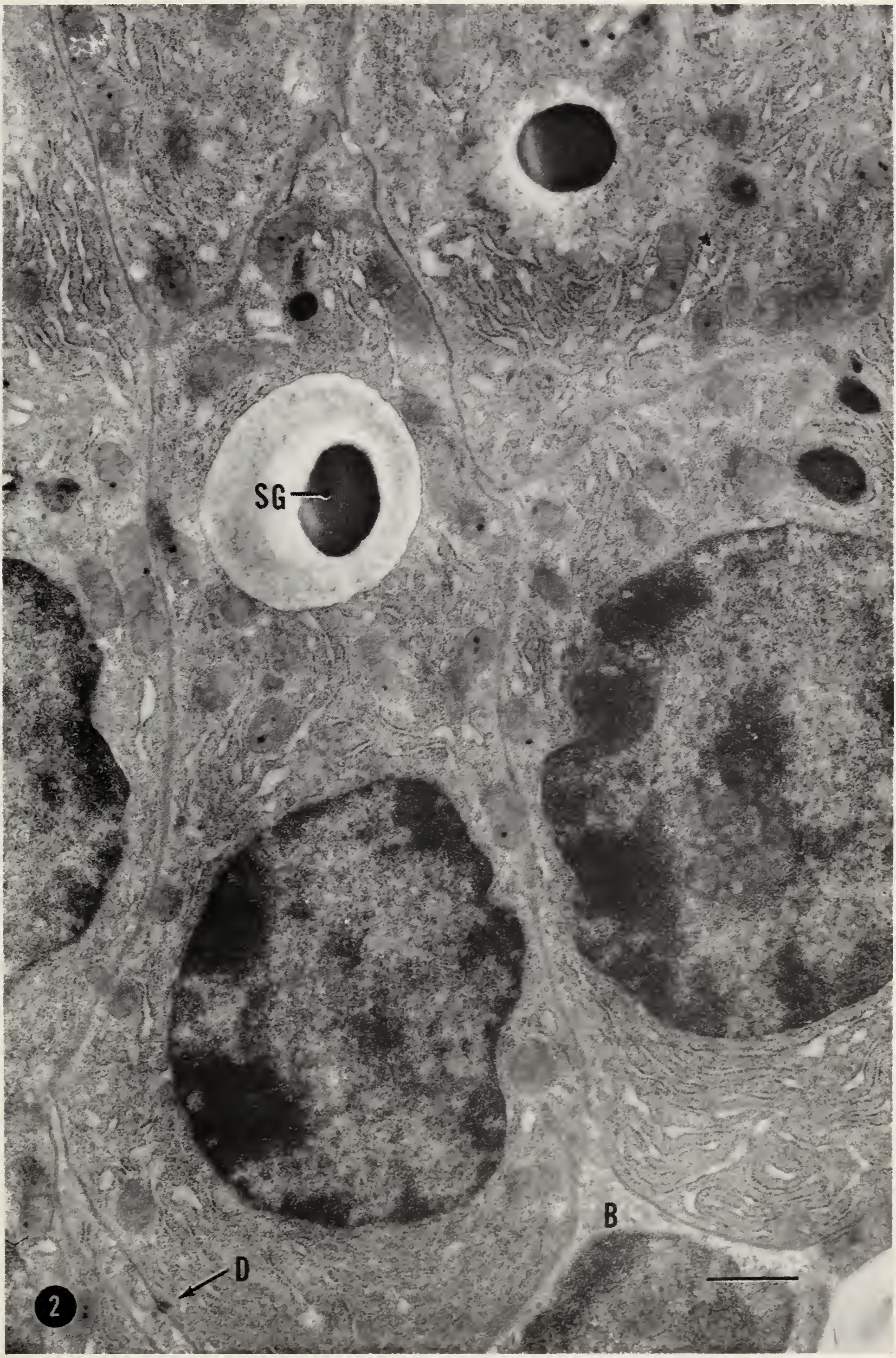
Figure 1.—Vertical section through seminal vesicle epithelium of normal young adult mouse. Most cells in this field are secretory, but at sites labeled B are portions of basal cells. C = capillary; CT = connective tissue space; Er = ergastoplasmic whorl; N = nucleus; M = mitochondria; G = Golgi complex, including large vesicles containing dense secretory granules; D = desmosome; Mv = microvilli; L = lumen, containing a few released secretory granules. Approximately × 7,500


L
M v
G
D
M
B
N
Er
B
CT
5 u
C
1

DEANE

Plate 12

Figure 2.—Slightly oblique section through seminal vesicle epithelium. B = basal cell; D = desmosome attaching a secretory cell to a process of a basal cell; SG = secretory granule within a large Golgi vesicle. Marker = 1 μ. Approximately × 14,000



DEANE

PLATE 13

FIGURE 3.—Vertical section through base of secretory cell and underlying capillary. *Note* smooth-surfaced microtubules (Mt) extending in from base of secretory cell at two points on base. Ergastoplasm (Er) consists of membrane-bound channels studded with ribosomes. Its channels sweep from the base toward the upper part of the cell (*compare* fig. 5). B = portion of a basal cell; C = capillary, exhibiting fenestrated endothelium; BM = basement membranes of epithelium and capillary; M = mitochondrion; DB = dense body, possibly a lysosome or mass of lipofuscin. Approximately × 24,000

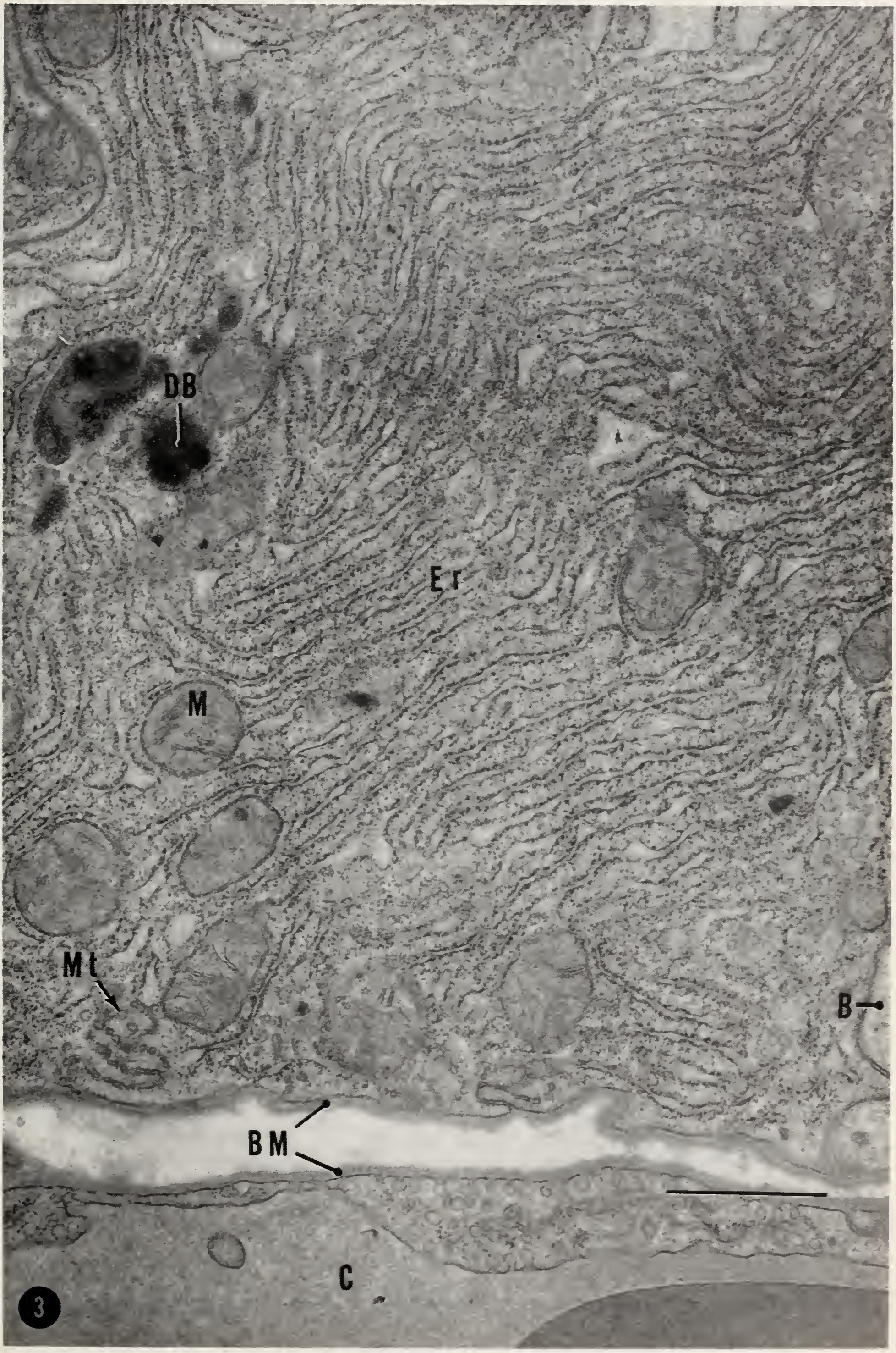

DB
Er
M
Mt
B
BM
C
3

Plate 14

Figure 4.—Cross section through secretory cell, made in basal half of cell. Especially evident is the concentric disposition of ergastoplasmic channels. B = process of basal cell; Mt = microtubules extending in from lateral surface of cell; N = tangential section of nucleus, showing its pores to good advantage. Approximately × 24,000

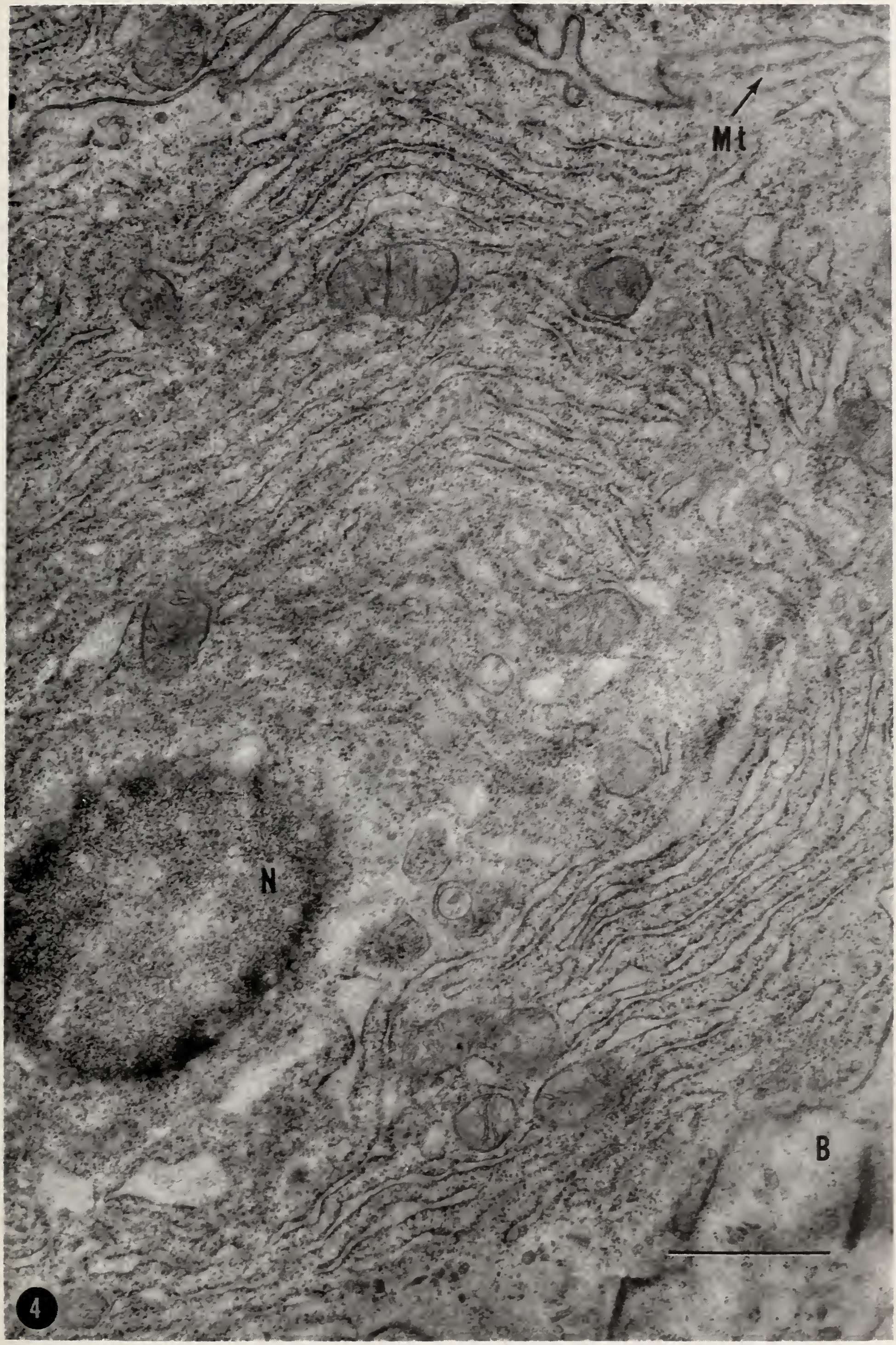

Mt
N
B
4

Plate 15

Figure 5.—Vertical section through secretory cells at apical region. Golgi complex consists of typical packets of flattened cisternae plus vesicles of various sizes. Within some flattened cisternae are ribbons of material with about the same density as that in the typical secretory granules in larger vesicles. Ergastoplasmic membranes approach Golgi membranes closely at many points, though continuity of channels is not definitely demonstrable. Other ergastoplasmic channels approach, but do not reach, apical surface of cell. GV = large Golgi vesicle containing a dense secretory granule surrounded by material of low density; GM = membranes of flattened Golgi cisternae; S = secretory material in small Golgi vesicles; Mv = microvilli on apical surface of cell. Approximately × 24,000

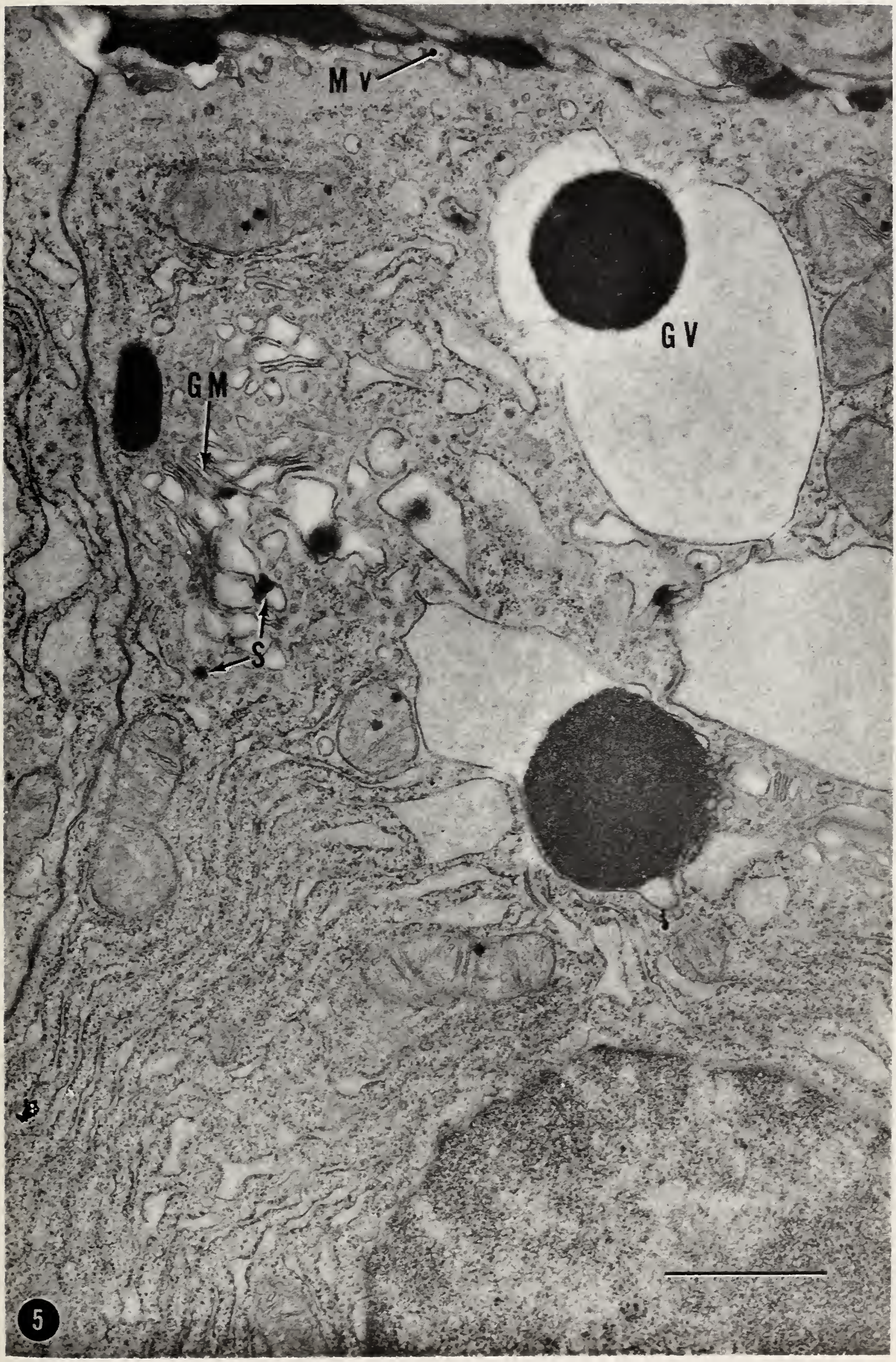

Mv
GV
GM
S
5

PLATE 16

FIGURE 6.—Tangential section through epithelium near its base, showing portions of several basal cells (#1 to 4) and several secretory cells. Basal cell cytoplasm contains numerous mitochondria, occasional ergastoplasmic strands (Er), scattered free ribosomes, some smooth membrane-bound channels that may represent agranular endoplasmic reticulum, and packets of smooth membrane-bound systems that appear to be the Golgi apparatus (? G). At P, a basal cell sends a studlike process into adjacent secretory cell. Approximately × 24,000

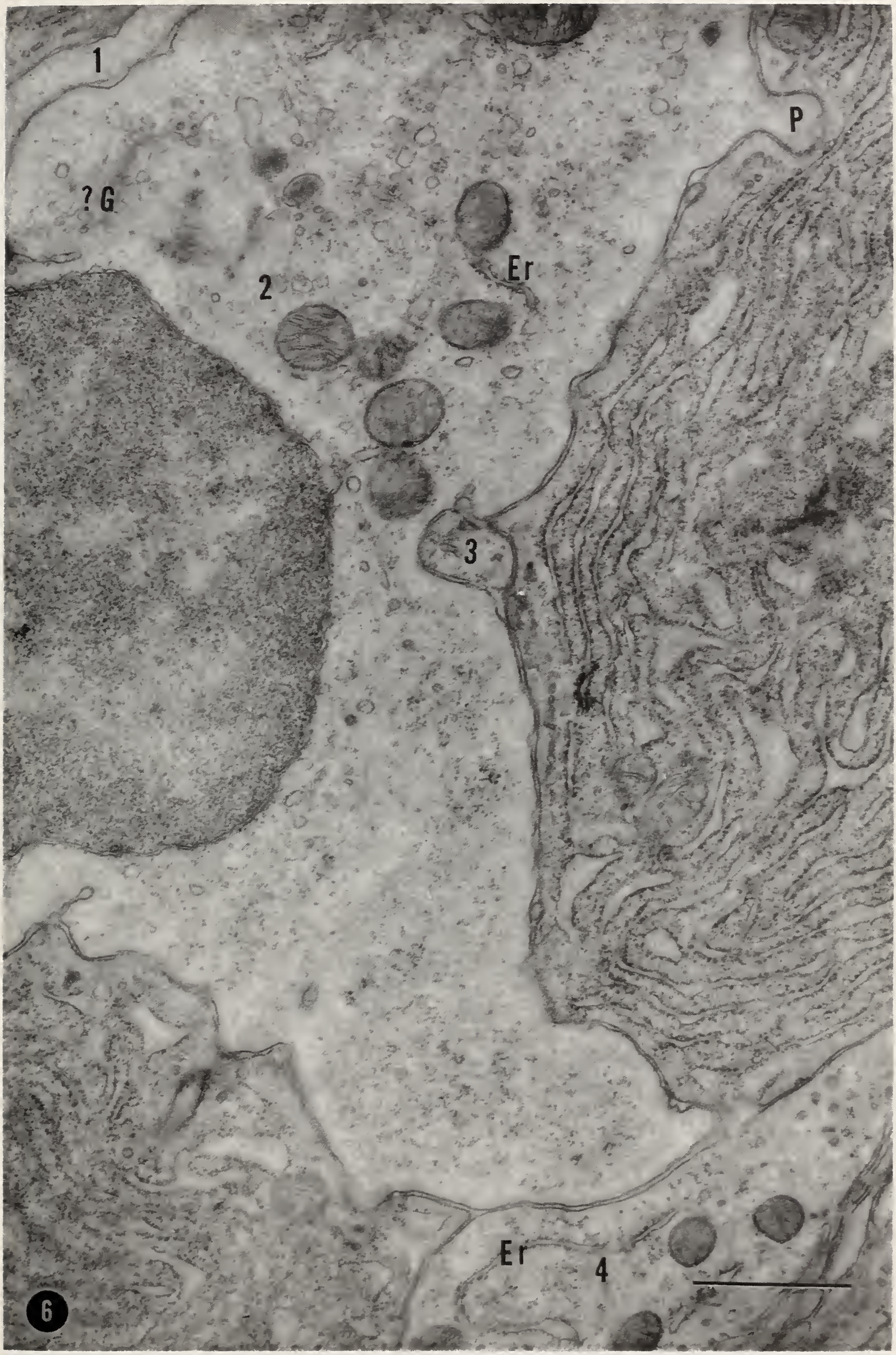
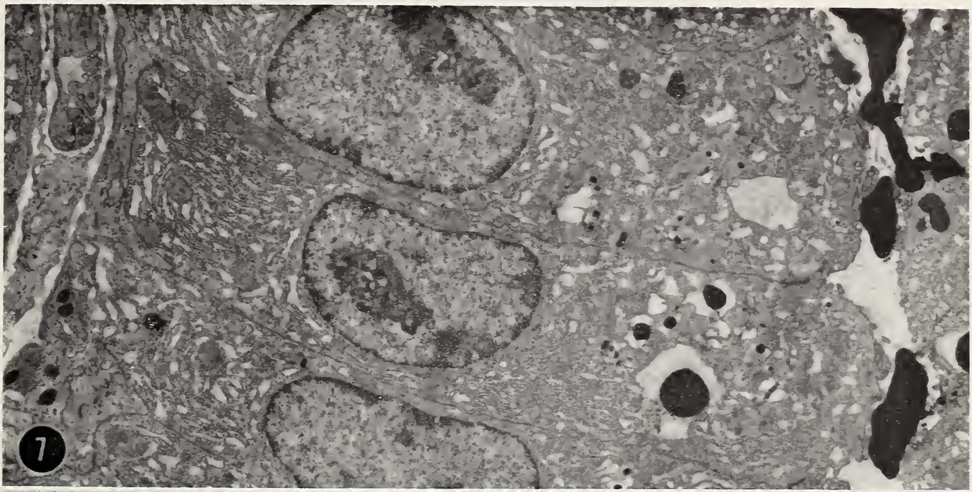

1
P
?G
Er
2
3
Er
4
6

Plate 17

Figure 7.—Vertical section through seminal vesicle epithelium from block fixed in 2 percent buffered OsO_4 without any additive. *Note* relative distention of ergastoplasmic channels. × 5,400

Figure 8.—Oblique section through seminal vesicle epithelium from block fixed in 2 percent buffered OsO_4 containing 20 percent sucrose (0.6 M). Ergastoplasmic channels are tightly closed. × 5,400

Figure 9.—Oblique section through seminal vesicle epithelium from block fixed in 2 percent buffered OsO_4 containing 2 percent NaCl (0.6 osmolar). Ergastoplasmic channels are not closed, though red blood cell in capillary to *upper left* is crenated. × 5,400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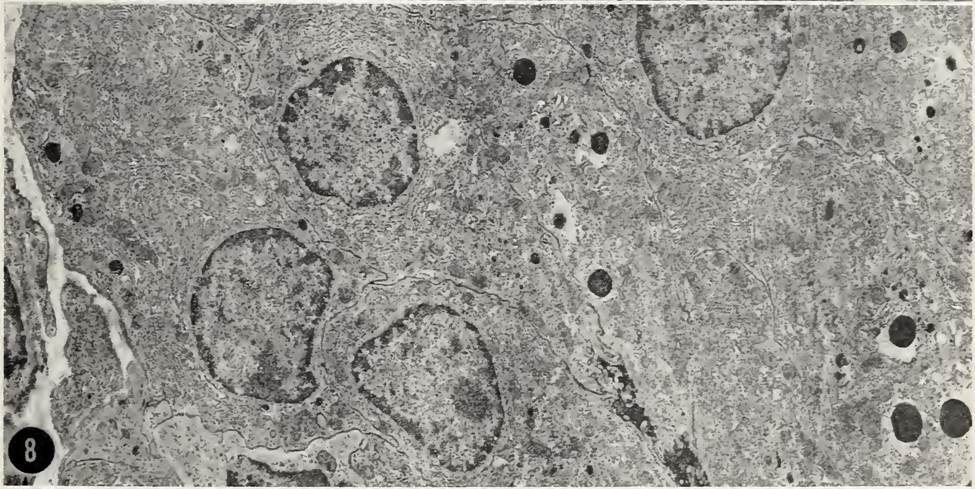
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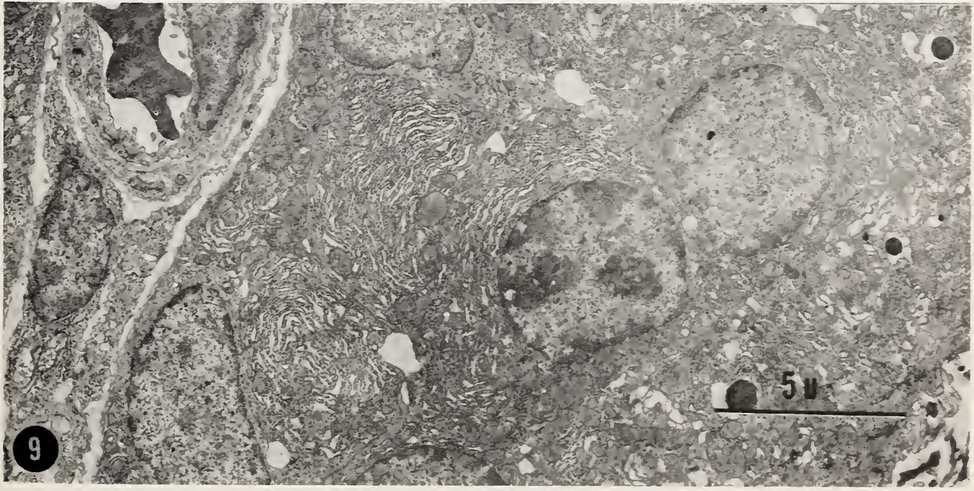
9
5 µ

PROSTATIC ULTRASTRUCTURE[1, 2]

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The androgenic dependence of prostate was first studied histologically and has been repeatedly measured in the rat. In the first definitive electron microscopic study of prostate (*17*), we therefore examined rat ventral prostate so we could compare our results with the well-documented reports of others using different techniques (*24, 28*). This report describes the ultrastructure of rat prostate in young, adult, and old animals; in castrates; and in castrates treated with testosterone before or after castration.

METHODS

Electron microscopic studies were made of ventral prostates of albino rats observed under these conditions: 1) normal, young adult rats weighing 175 to 200 g; 2) normal, sexually immature, weanling animals weighing 50 to 70 g; 3) normal rats, at different ages, weighing 100, 150, 200, 300, 400, 500, and 600 g; and 4) young adult males weighing 175 to 200 g castrated and examined 1, 2, 4, 8, 16, and 32 days later. Prostatic fragments, 1 mm in diameter, were fixed in Dalton's chrome-osmic acid solution (*8*), osmic acid with sucrose (*7*), and in Luft's permanganate solution (*22*). Caulfield's osmic acid and sucrose fixative was useful for demonstrating the granular component of the endoplasmic reticulum; however, with this treatment we observed numerous interruptions in the continuity of plasma membranes. With permanganate fixation, membranes were well preserved, but the degree of dilatation of the ergastoplasmic sacs was inconstant. In these studies tissues were embedded in methacrylate.

Testosterone treatment and castration.—Fifteen male Wistar white rats weighing 190 to 200 g were castrated. The rats were divided into 3 groups. Each rat received 7 daily injections of 1 mg of testosterone propionate in oil. Animals in each group were started on the testos-

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] Supported in part by U.S. Public Health Service grants C-2836 and NB 04330-01.

terone injections at 3 days prior to castration, 4 days after castration, and 8 days after castration. Prostatic fragments were fixed in osmium tetroxide with sucrose or in Luft's permanganate fixative and embedded in methacrylate.

Supplemental studies.—Later, ventral prostates from 4 additional rats were examined. The tissue was fixed in a modified Dalton's chrome osmium solution and embedded in Maraglas epoxy resin (*15, 20, 27*). The modified fixative was prepared by adding 5.4 mg of calcium chloride to each ml of prepared Dalton's fixative. Two animals were untreated. Two had been castrated and were killed after they had received 7 daily injections of 1 mg of testosterone propionate, the treatment starting on the 8th day after castration.

RESULTS

Normal Adult Rats

In the ventral prostate of the mature rat the epithelial cells are tall and columnar. The luminal surfaces of these cells bear varying numbers of microvilli (figs. 1 and 2). Apical sacs of the epithelial cells contain droplets or granules that are only slightly more electron-dense than the adjacent endoplasmic reticulum (fig. 2). Some variation exists in shape, diameter, and size of these "secretory" droplets. The pale, apical secretory granules seen in electron micrographs of rat ventral prostate are different from the very dark globules in epithelial cells of dorsal prostate of the rat and in the seminal vesicle epithelium of the mouse (*4, 10, 12, 13, 29, 30*).

In epithelial cells of normal adult prostate a large Golgi complex lies in the supranuclear region of the cell midway between the nucleus and the apex. In the supranuclear region immediately adjacent to the nucleus, the ergastoplasmic sacs are arranged in a pattern of concentric lamellae. The outer nuclear membrane is continuous with the membranes of the ergastoplasm (figs. 3 and 4) (*16, 18, 19*).

Immature Rats

Prostatic epithelial cells of sexually immature rats are much smaller than those of mature animals. Only a small cytoplasmic volume is occupied by ribosomes and ribosome-covered sacs in the epithelium of the immature rat. The Golgi complex of the epithelial cells is well developed (*19*).

Older Animals

Prostatic epithelial cells in large old rats are patterned much the same as those in young sexually mature animals. The feature distinguishing epithelial cells of older animals is supranuclear lipide. Size of deposits increases with age. The largest lipide bodies are found in the supranuclear region at the core of concentrically arranged ergastoplasmic lamellae (*18, 19*).

Castrated Rats

If adult rats are castrated, the prostatic epithelial cells become much smaller than normal. The striking reduction in cell size that takes place in the first weeks after castration appears to result, in large part, from the extreme collapse of ergastoplasmic sacs. The postcastration collapse and loss of ergastoplasmic sacs is most prominent in the part of the cell nearest the lumen of the gland. There is also a reduction in number of ribosomes and mitochondria and the remaining mitochondria are smaller. Large electron-dense lipide bodies are found in the supranuclear zone of epithelial cells of castrates. A variation in electron density is seen within these bodies, which suggests an internal structure. Other globules of electron-dense material are found within or adjacent to some of the membrane-lined sacs in the Golgi region (*17*).

Castration and Testosterone Treatment

In testosterone-treated castrate adult rats, if the treatment is started within 5 days after castration the epithelial cells maintain a structure similar to, and in some instances indistinguishable from, that of normal adult control animals. The ribosome-covered sacs of the endoplasmic reticulum appear as distended as in normal adult animals. Secretory "granules" are also found in treated castrates. Lipide particles and myelin figures are sometimes found in the cytoplasm of epithelial cells of treated castrates (figs. 5 and 6). Similar fat particles are also found in epithelial cells of the untreated castrates. Animals in which testosterone treatment is started prior to castration sometimes have prostatic epithelial cells with quite distended ribosome-covered sacs. It is not known whether this distention of sacs was a change present in the living cell or one caused by the vagaries of fixation and preparation of the tissues. We have not repeated our earlier experiments on testosterone treatment followed by castration with our present techniques of tissue preservation for electron microscopy. We have repeated the experiments on castration followed 8 days later by 7 daily testosterone injections with the use of improved methods for fixation and embedding of tissues; results were much the same as in our earlier study (figs. 5, 6, and 7).

COMMENT

The epithelial cells of the prostate of the rat have very prominent cytoplasmic ribosome-covered sacs. If androgenic stimulation of the prostate is absent or markedly diminished, such as in the sexually immature animal or in the castrate, the granule-covered cytoplasmic sacs are collapsed and the ribosome granules sparse. Testosterone treatment started soon after castration restores the prostatic ultrastructure to normal. In electron microscopic studies of prostatic epithelial cells, changes in androgenic hormonal stimulation are paralleled promptly, consistently, and dramatically by changes in the ergastoplasmic sacs. The contents of these sacs are believed to represent precursors of cellular secretion.

Cytoplasmic lipide-containing structures are prominent in prostatic epithelial cells of castrates and older animals. We have observed, as have others, that there are several morphological types of cytoplasmic lipide bodies in epithelial cells (*9, 17–19, 21, 25, 26*). In our studies we have examined epithelium of prostate, kidney, skin, and lungs, as well as fibrous tissue, bone, peripheral nerves, brain, heart, and skeletal muscle (*20*). Some cytoplasmic electron-dense structures appear to be lipochrome, ceroid, and other pigments (*9, 17, 19*). Some cytoplasmic lipide bodies contain enzymes. On the basis of correlated electron microscopic and biochemical studies, Novikoff, DeDuve, Miller, and others (*14, 23, 25, 26*) have found cytoplasmic lipide-containing, membrane-lined structures containing hydrolytic enzymes; such structures were named lysosomes. Some of the lipide structures in prostatic epithelial cells may be lysosomes, but, others that differ structurally possibly also differ functionally and are fat deposits or organelles of an undetermined character (*1–6, 9, 11*).

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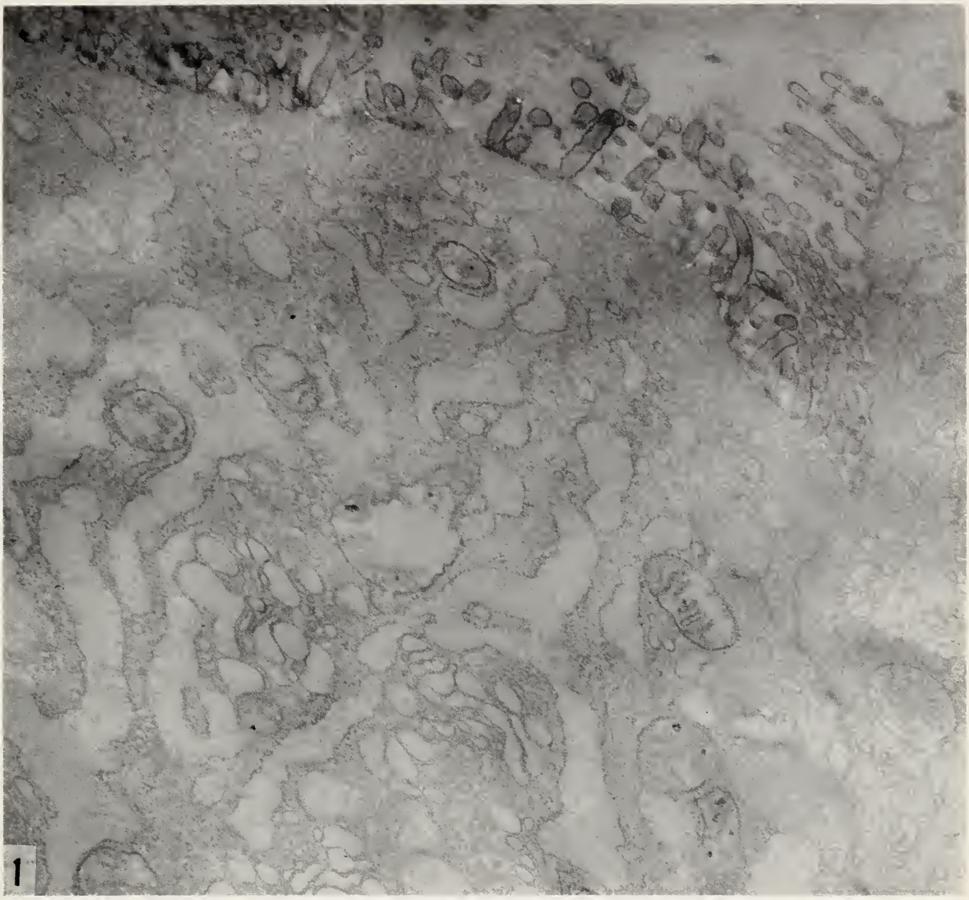
PLATE 18

FIGURE 1.—*Normal.* Electron micrograph of apical regions of 2 epithelial cells from the ventral prostate of a normal 190 g young adult rat. Prominent Golgi complex in *lower* part of picture. At *top,* microvilli can be seen projecting into the lumen. All illustrations are of tissue fixed in modified Dalton's osmium tetroxide solution and embedded in Maraglas epoxy resin. Approximately × 30,000

FIGURE 2.—*Normal.* "Secretory" granules of low-electron density lie within membrane-lined sacs. Number of apical microvilli varies in normal epithelial cells. Approximately × 40,000

1



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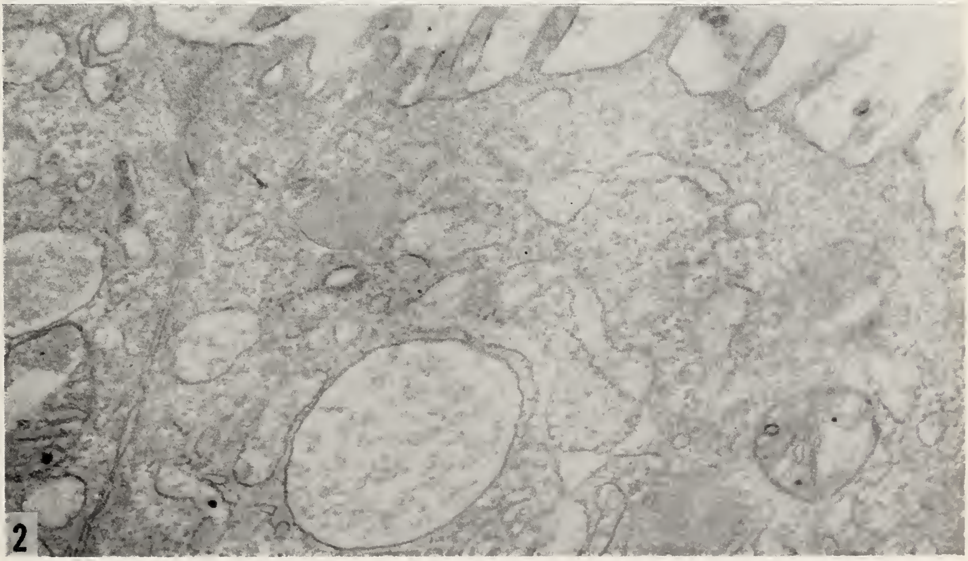
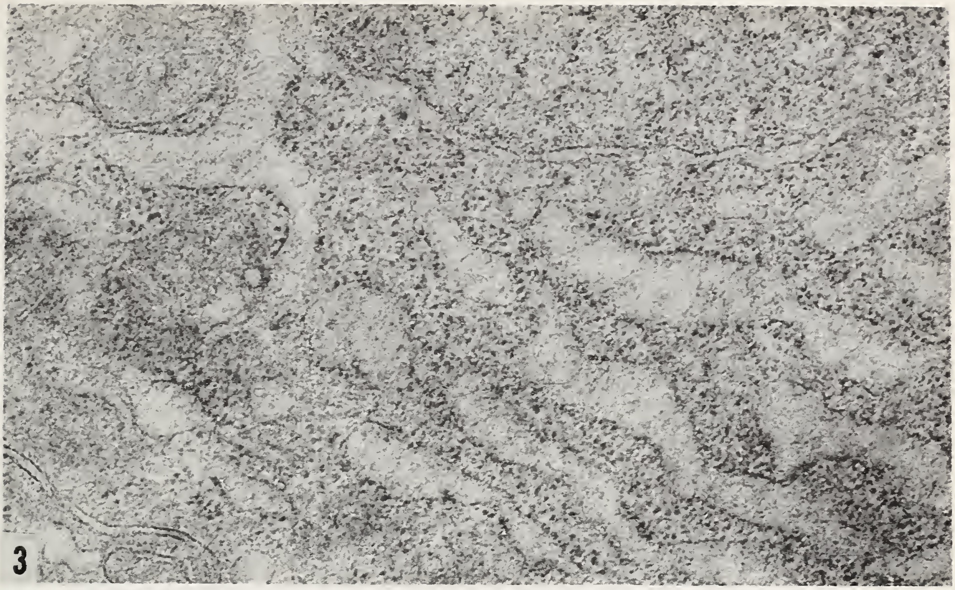


HARKIN

Plate 19

Figure 3.—*Normal.* A small part of nucleus and adjacent endoplasmic reticulum are illustrated. The degree of distention of ergastoplasmic sacs represents that consistently found in normal ventral prostate fixed in modified Dalton's fixative, as outlined in text. Approximately × 55,000

Figure 4.—*Normal.* At *left* a small part of an epithelial cell is seen. Paralleling the plasma membrane of the epithelial cell is the basement membrane, which is less electron-dense or discrete than the plasma membrane at cell surface. There are scattered collagen fibrils between cells. Cell processes of 2 fibrocytes are in *center.* At *right* is a smooth muscle cell. Approximately × 50,000



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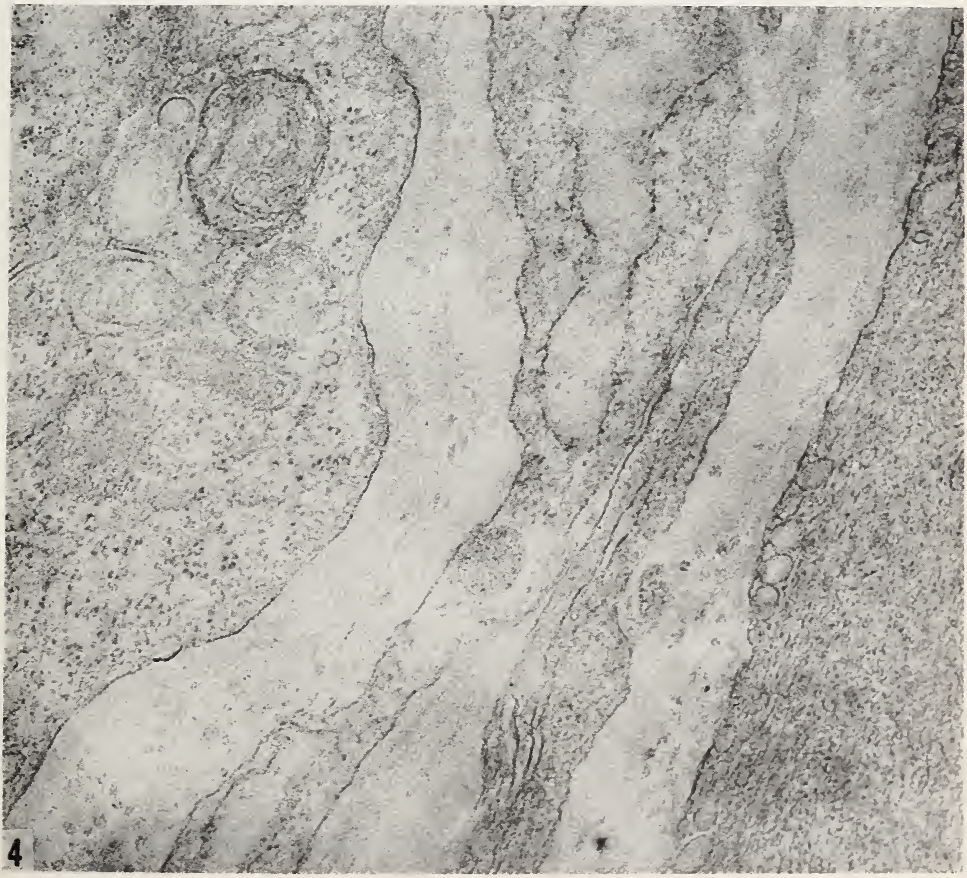

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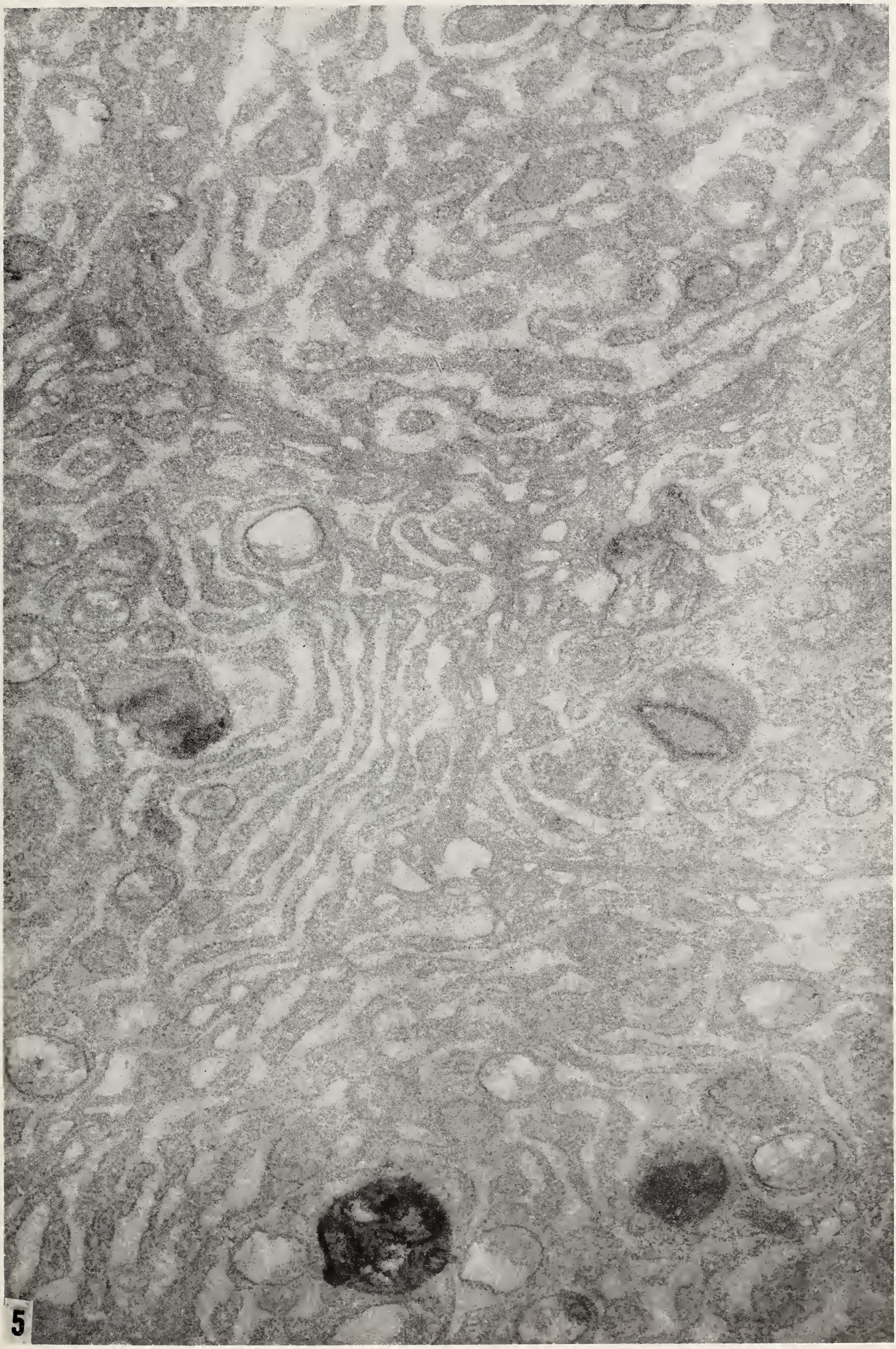
PLATE 20

FIGURE 5.—*Testosterone-treated castrate.* The micrograph includes part of the supranuclear zone of 4 epithelial cells. Distention of the ergastoplasmic sacs appears similar to that observed in cells of the normal control rats. Five electron-dense bodies are present. In the body to the *left* of *center* no internal structure is present except for a variable density or ripple, a pattern often observed in sections of relatively homogeneous lipide. The electron-dense body to the *right* of *center* contains internal membranes. Large, dense body at the *bottom* of illustration has a more complex internal structure than that of the two described above. Approximately × 25,000



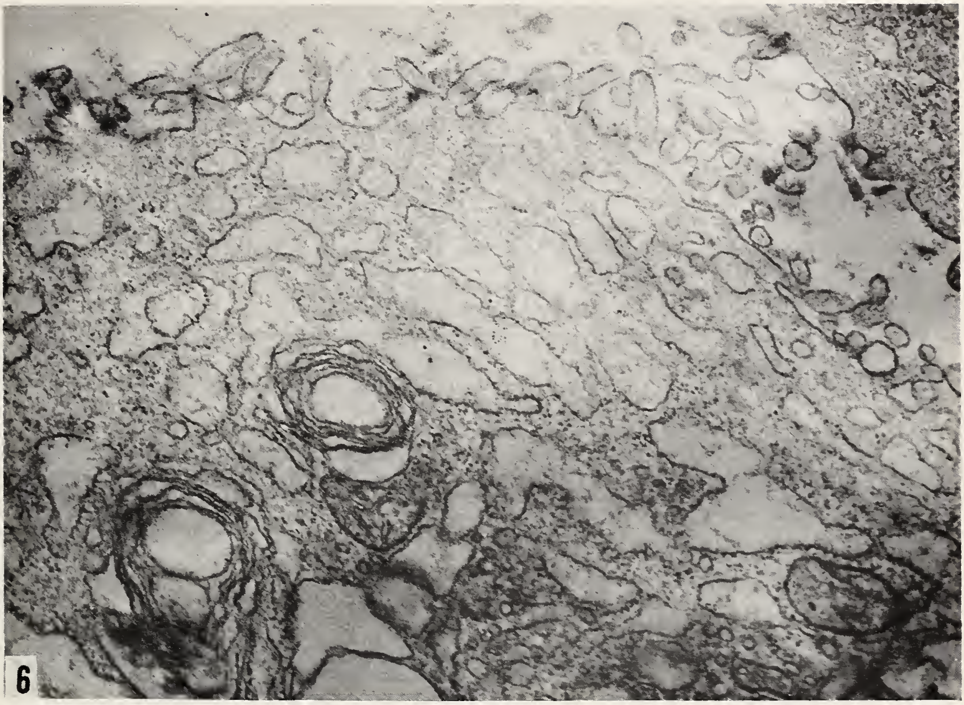
5

HARKIN

PLATE 21

FIGURE 6.—*Testosterone-treated castrate.* In the apical cytoplasm, myelin figures are found in membrane-lined spaces. Approximately × 35,000

FIGURE 7.—*Testosterone-treated castrate.* The smooth muscle cell has numerous cytoplasmic projections at the surface. Such a surface configuration of muscle cells is often prominent in castrates, with or without testosterone treatment, but is also seen in normal animals. Approximately × 30,000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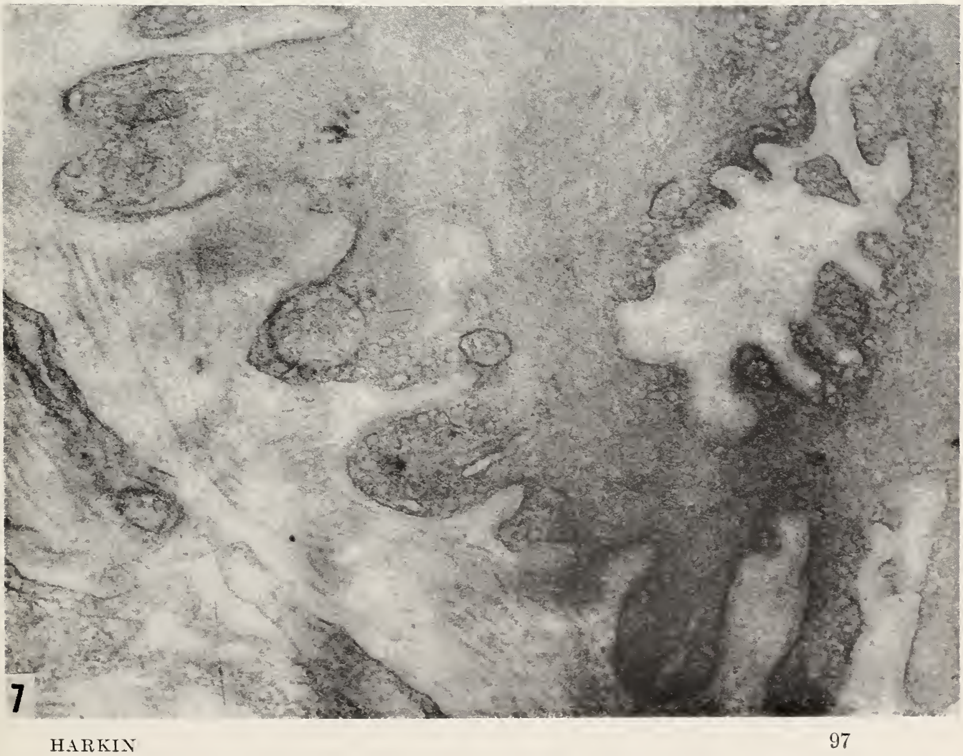
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DITHIZONE CHANGES IN RAT LATERAL PROSTATE[1]

G. RANDOLPH SCHRODT, Department of Pathology, University of Louisville, School of Medicine, Louisville, Kentucky

REFERENCE has been made to a number of structural and biochemical differences between various lobes of the rat prostate. A most interesting difference is the level of zinc found in these different lobes. Zinc is richest in the lateral lobe. Histochemical and radio-zinc methods indicate the greatest concentrations at the apex and base of the epithelial cell. Some zinc has been shown to be an integral part of certain metal enzymes, though most of it has not been defined biochemically. When the metal chelating agent, dithizone, is administered to rats there is a rather selective necrosis of the lateral portion of the prostate with little or no change in the other lobes. The ultrastructural changes in the lateral prostate of the rat after the administration of dithizone are shown in figures 1, 2, 3, and 4.

Figure 1 shows the apical portion of lateral prostate epithelium. There is a rather prominent development of the microvilli in these epithelial cells. Also note some vacuoles with electron-dense bodies similar to those described by Dr. Deane in the seminal vesicles. There are mitochondria and a fairly well-developed endoplasmic reticulum.

The first changes described with the light microscope following administration of dithizone appear in about 3 hours. We have seen focal and rather minor changes in about ½ hour, and these consist of some loss of the microvilli and the deposition of electron-dense material over the apical cytoplasm.

In 3 hours, in addition to the loss of microvilli there is some clearing of the apical cytoplasm and some loss of the electron-dense granules in the Golgi zone. A similar change is seen at the basal portion of the epithelial cells, and where this occurs, the epithelial cells separate from the underlying stroma. Electron-dense material often accumulates at the site of separation (fig. 2). A few hours later most of the epithelial cells have sluffed into the lumina and appear necrotic. The stroma remains essentially unaltered (fig. 3).

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

Following this almost total destruction, regeneration is noted in about 3 days. At the end of the 1st week there is some development of microvilli, but not to the degree seen in the untreated animals. The Golgi zone is present in the epithelial cells but is not as prominent as in the untreated animals. At 3 weeks the lumina are still filled with necrotic debris. Later the debris clears and the epithelium returns to "normal" (fig. 4).

The early changes in the epithelial cells after administration of dithizone occur in the areas of the cell where zinc concentration is high, namely the apical and basal portions. This finding lends support to the idea that the cell necrosis is perhaps due to chelation of zinc by dithizone.

There have been a few clinical trials with dithizone in the treatment of prostate carcinoma. Perhaps at one of the later conferences some of the clinicians who have had experience with this agent may have additional comments.

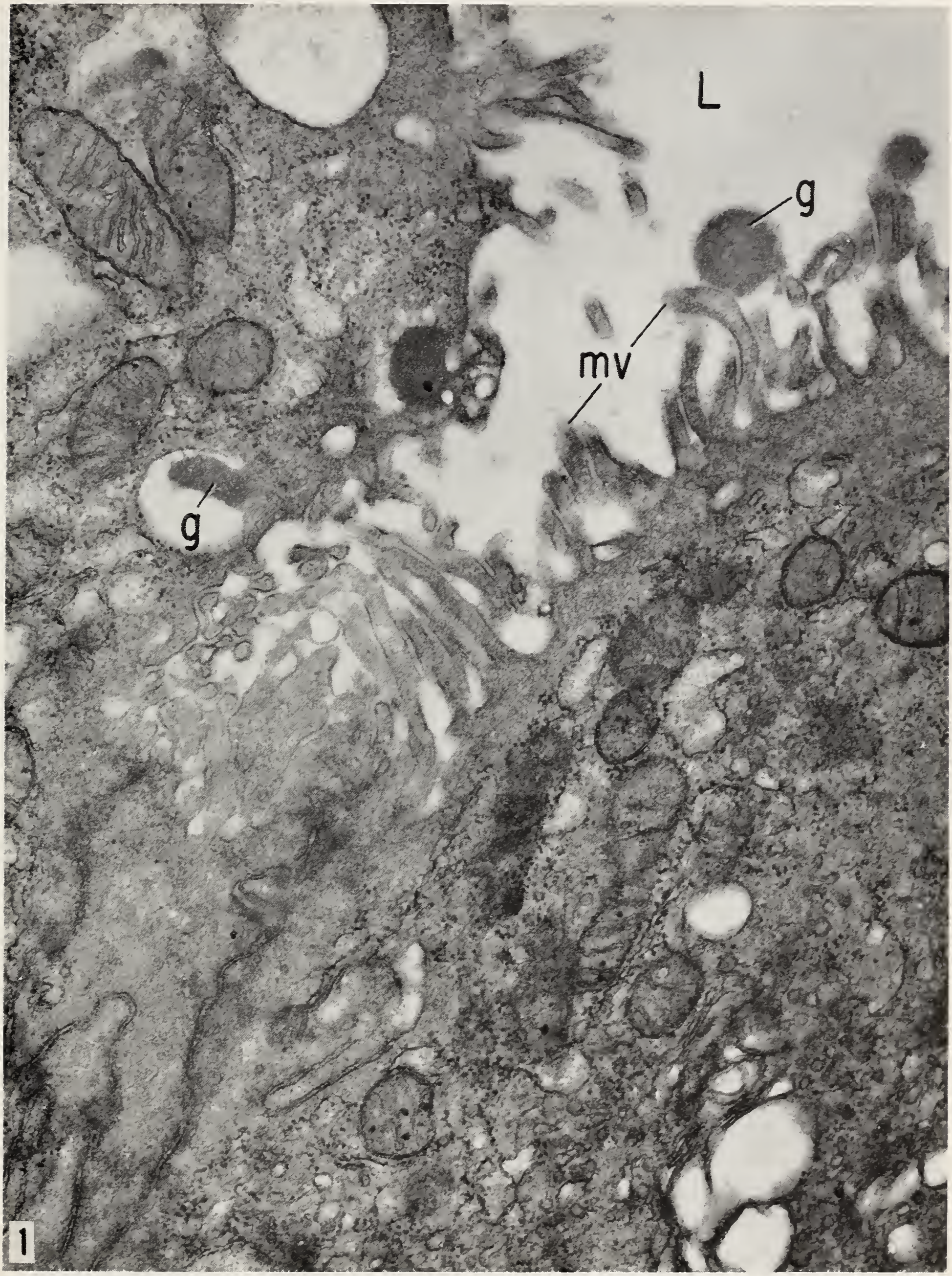

FIGURE 1.—Normal lateral rat prostate epithelium. Apical portion of two epithelial cells are seen. Microvilli (mv) are tall. Electron-dense granules (g) are noted in large vacuoles in the apical cytoplasm and lying free in lumen (L). × 45,000

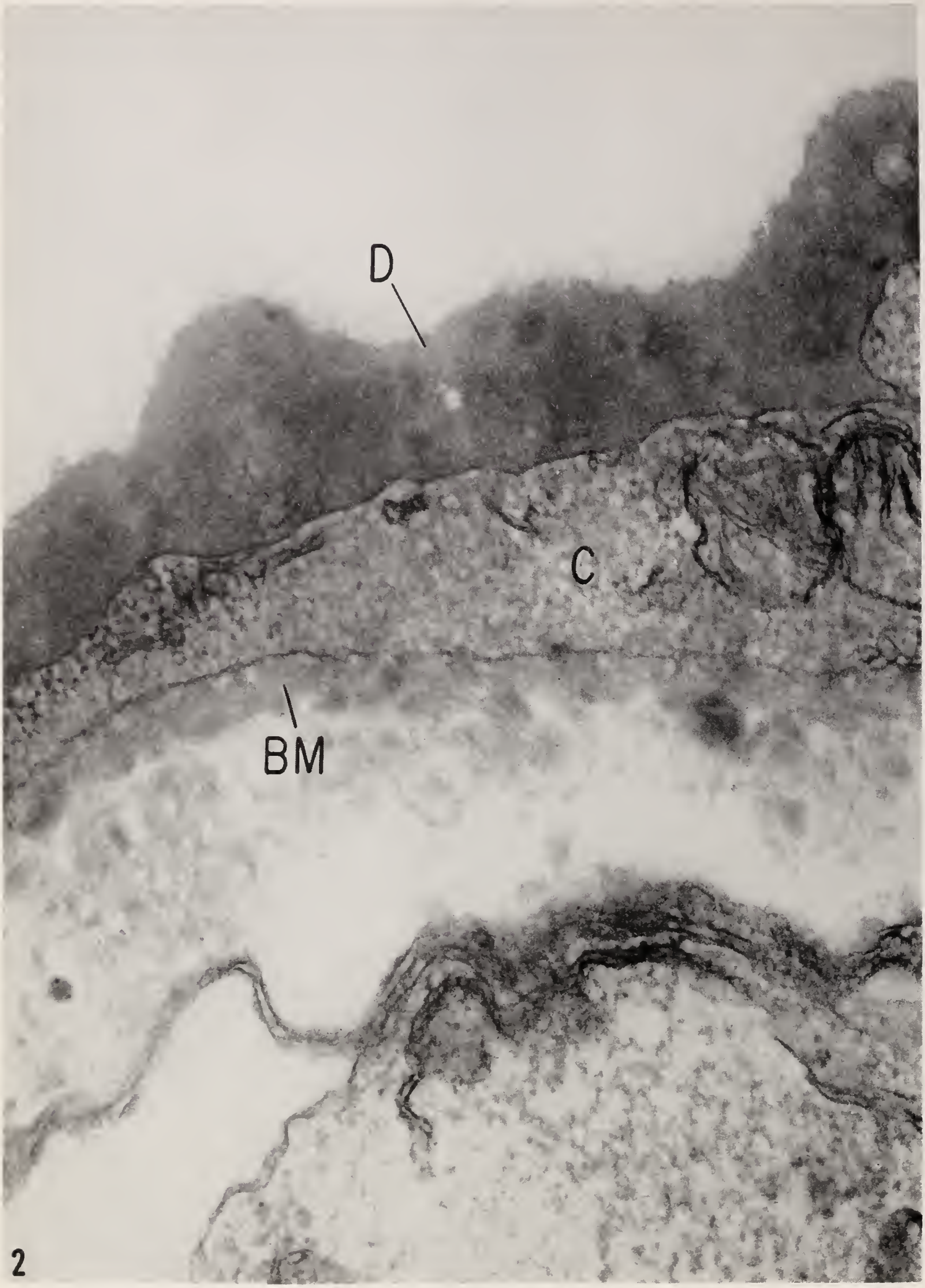

FIGURE 2.—Lateral prostate 3 hours post dithizone, showing a layer of electron-dense material (D) overlying basement membrane (BM). Portion of a "reserve cell" (C) lies between the dense material and basement membrane. × 79,000

SCHRODT

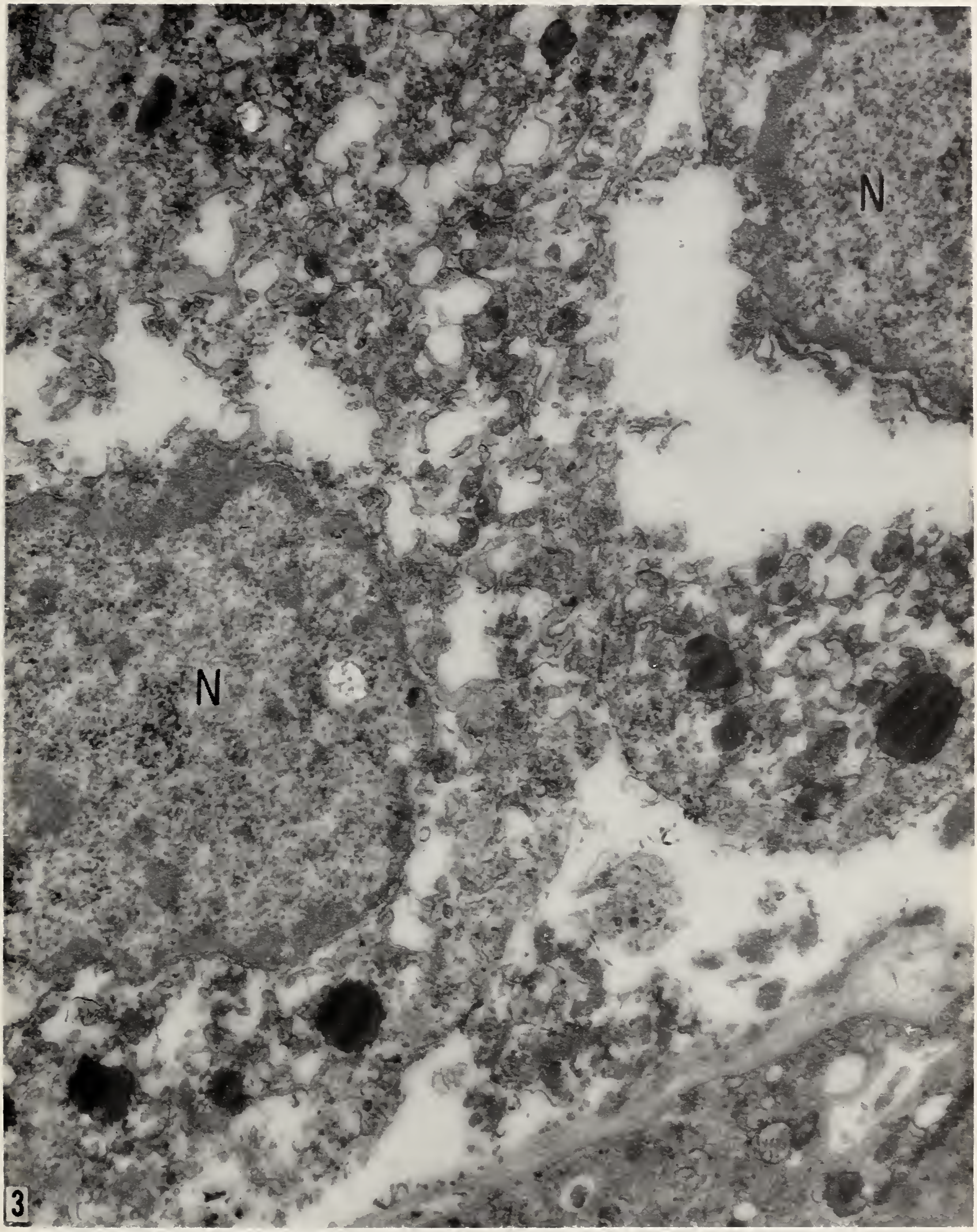

FIGURE 3.—Necrotic epithelial cells from rat lateral prostate 6 hours post dithizone Cell organelles are poorly defined. Nuclei (N) are discernible but show periphera clumping. × 17,500

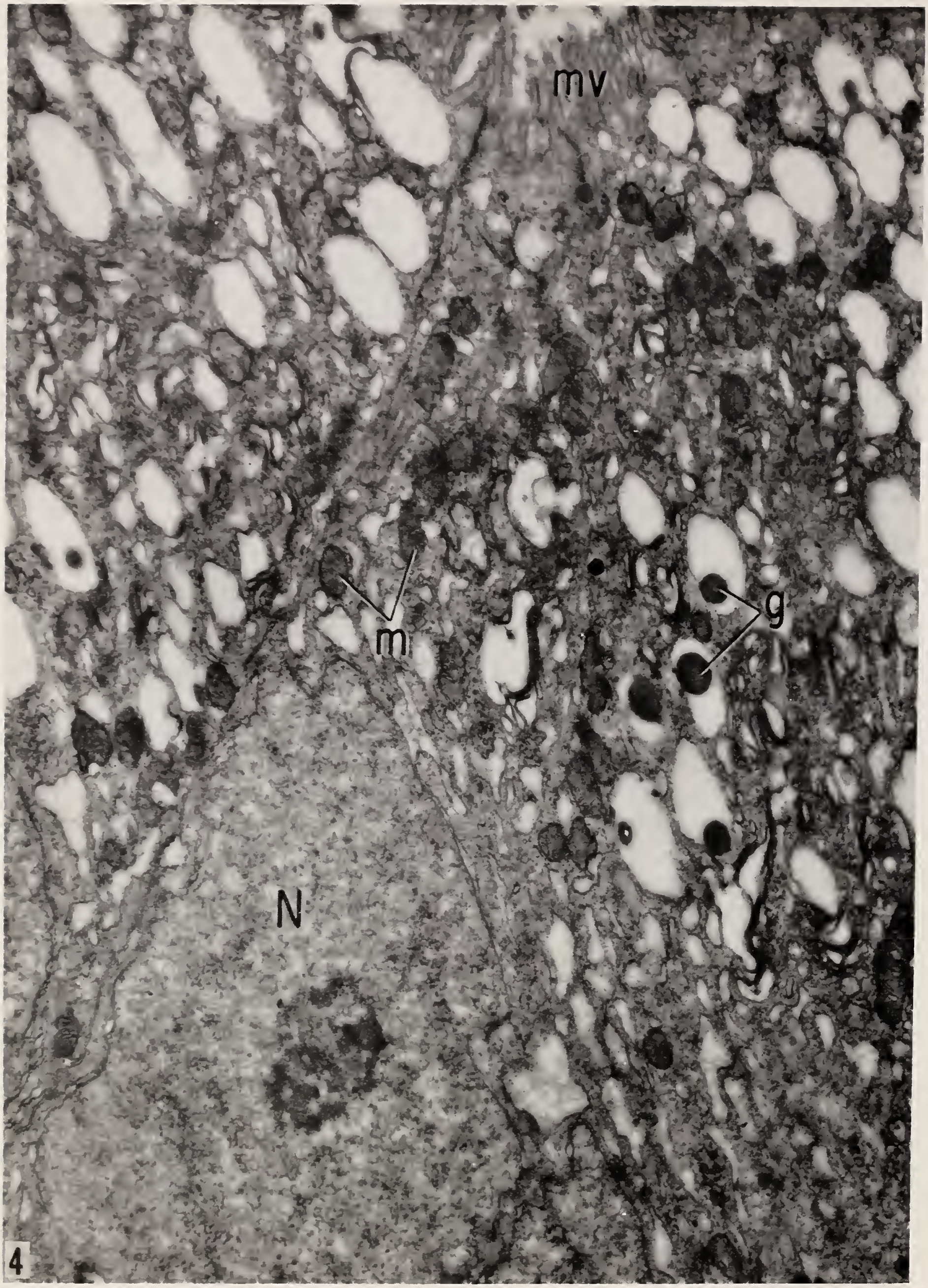

FIGURE 4.—Lateral prostate 7 weeks post dithizone. Cells appear normal. N= nucleus; mv = microvilli; g = Golgi granules; m = mitochondria. × 19,000

SCHRODT

DISCUSSION

Dr. Mann: One of the puzzling biochemical features of the secretory fluids produced in the prostate and other male accessory glands is the presence in these fluids of certain enzymes that normally occur in high concentrations within intact cells but not in the animal body fluids. The mammalian seminal plasma, which represents the combined accessory secretions, is, for example, rich in flavoproteins, transaminases, and intermediary enzymes of glycolysis, *e.g.*, phosphohexose isomerase and aldolase (zymohexase). Is it possible that some of these proteins are carried into the secretions not as soluble secretory products of the epithelial cells in accessory glands, but attached to some of the characteristic electron-dense structural cellular elements which pass from the secretory cells, unchanged, into the lumen of the glands? Have you observed in the secretion of the mouse seminal vesicle any clear signs of cellular desquamation or the presence of any element normally also present in the cytoplasm of the intact epithelial cells?

Dr. Deane: In normal mice I have never seen fragments of cytoplasm out in the lumen. In the seminal vesicles of weanling mice I have seen mitochondria and other debris out in the lumen that suggested desquamation of cells. Whether at the time of ejaculation certain cells are desquamated, which would not appear free in preparations fixed with my method, I do not know.

Dr. Brandes: In answer to a question of Dr. Mann's I would like to say that in the ventral prostate of the mouse there appears to be an apocrine mechanism of secretion whereby the apical portion of the cell becomes homogeneous and constricted, and finally is detached. Many times the cells include secretory granules and mitochondria. In the rat the mechanism of secretion appears different. The secretory granules fuse with the plasma membrane, and finally they seem to become detached into the lumen.

Dr. Brendler: The mitochondria have been mentioned in some of these presentations, but no one has offered any information as to whether they decrease in number or size after castration. I have recently seen a publication by a Japanese investigator, Kanai, showing that mitochondria do not actually decrease in number, but rather in size. Would anyone comment on this?

Dr. Deane: In the mouse 4 weeks after castration there does not seem to be a decrease in number of mitochondria per cell. In consequence, mitochondria appear much more concentrated in the cell, and there is much less cytoplasm. I think they are smaller, just as they are smaller in basal cells than in active secretory cells. But they do not look less complex.

Dr. Jensen: I was especially interested in Dr. Bourne's illustration that, in the castrated animal, the administration of testosterone partially, but not completely, restored the chemical picture to normal. Is there any evidence, besides this chemical evidence in the castrated animal receiving testosterone, that the prostate is not quite the same as the normal prostate? Certainly, from the work of Huggins and Scott on the castrated dog, the administration of testosterone restored the ability of the gland to secrete the prostatic fluid at, or even in excess of, the normal level.

Dr. Kochakian: I would like to ask Dr. Bourne what his treatment was. Maybe he did not treat the animals long enough, or the dose was not large enough to bring the prostate back to normal.

Dr. Brandes: There were very short periods of recovery.

Dr. Bern: To comment further on the basal cell issue that Dr. Deane raised, I do not know where other secretory cells could come from except by the mitotic proliferation of undifferentiated cells. This would seem the most reasonable function to attach to the basal cells. Furthermore, the basal cells also appear to be the source of the metaplastic epithelium that occurs in the mouse seminal vesicle after estrogen. This raises again the point that Dr. Price mentioned regarding the ability of estrogen to stimulate mitosis in the seminal vesicle. In the metaplastic response the basal cells undergo mitotic proliferation, but this does not mean that the estrogen is mimicking androgen.

May I ask Dr. Schrodt whether his observations give any information as to where the basal cells are and what they are doing.

Dr. Schrodt: I noticed them in the lateral lobe and have no further comment.

Dr. Rosenkrantz: In regard to what Dr. Bern just said, we do have some biochemical evidence indicating that estrogens will mimic androgens in the seminal vesicles. Using the tetrazolium method, we compared the effects of androgen and estrogen on the succinic dehydrogenase levels of the prostate and seminal vesicles. We found, in the castrated rat given estradiol-17β or diethylstilbestrol, there is an increase in succinic acid dehydrogenase in the seminal vesicles.

I would like to ask Dr. Bourne a question. On the acid phosphatase slides shown in relation to prostatic carcinoma, I could not detect whether there was an increased deposition of acid phosphatase around the capillaries. This is of interest since the route of leakage of presumptive prostatic acid phosphatase appearing in the serum remains unknown.

Dr. Bourne: I do not think these preparations demonstrate any increase around the capillaries, but possibly if the enzyme does leak through the capillaries, the histochemical method is not sensitive enough to demonstrate it.

Dr. Deane: On this point, the histochemical method is not useful for demonstrating enzymes which would go into solution in the medium. Certainly the enzymes that leave the cells and go into the vascular system would be in the soluble state.

Dr. Bern: The mimicking of androgens by estrogens may exist in the ability of estrogen to maintain a differentiated epithelium to some extent, but this is a little different from the stimulation of mitosis. We have obtained morphologic evidence of the ability of estrogen to maintain prostatic epithelium in the castrated rabbit in somewhat the same manner as androgen does.

Dr. Brandes: As to the leakage of acid phosphatase, the experimentally induced carcinomas were of the squamous cell variety and had already lost their acid phosphatase content.

Dr. Jensen: A question for Dr. Schrodt: Does the administration of testosterone simultaneously with your chelating agent in any way protect the prostatic cell from this deterioration?

Dr. Schrodt: We have not done that yet.

Dr. Mann: Years ago, during the study with Keilin which led us to the discovery of zinc in carbonic anhydrase and the identification of the first zinc protein as an enzyme, we observed frequently that certain metal-complexing agents, which are also inhibitors of that enzyme, interact with the zinc-protein complex as a whole. That is, they act without functioning as true chelating agents, which are capable of detaching the metal from the protein. We also observed that inactivation of the enzyme occurred not because it had lost the zinc, but because its protein component had undergone denaturation within the zinc-protein complex as a whole. It seems to me that the cellular degeneration changes, which you have observed in the rat dorsolateral prostate after an injection of dithizone, may have been due, in the first instance, to denaturation changes within zinc proteins as a whole, followed by loss of these metallo proteins together with the damaged cells, and not to a specific loss of *free* zinc as such, detached from the cells by dithizone.

Dr. Harkin: On the earlier question of the reversal of the changes following castration by the administration of testosterone, our particular studies were made on ventral prostates of 190 to 200 g rats. The animals received daily injections of 1 mg testosterone propionate in oil 4 or 8 days after castration. Each rat was treated for 7 days and then killed. Under these conditions, the ultrastructure of the epithelial cells of rat ventral prostate was reconverted essentially to that of uncastrated control animals.

Dr. Kochakian: It might be of interest to the group that in the kidney, androgen administration produces an increase in size of the mitochondria which seems roughly proportional to the increase in size of the kidney. This was pleasing to us because we had demonstrated earlier that the enzymes of the Krebs cycle also increase in proportion to the size of the kidney.

On this question of treatment, I think we must bear in mind, with respect to the effect of androgens on most of the metabolic and growth changes in the animal, that there are really two types of responses: There is a period of accelerated growth and then one of containment. So one would expect the type of changes in the two periods to be different.

Dr. Farnsworth: Several years ago Dr. Harkin had a paper in *Endocrinology*, reminiscent of some of the things that Dr. Brandes discussed. I noticed in both of these presentations that even after castration there seemed to be a congestion in the prostate and an enlargement of the endoplasmic sacs, as if when the androgen was taken away there was a production of secretion that could not get out. Then, a day or so after castration, there was a breakdown of the acinar border and a release of this material. I was wondering if this was not suggestive that the mechanism of androgen action might be to promote release of the secretion from the acinar border of the cells.

Dr. Harkin: After making the observation, as has Brandes, I did speculate as to the cellular site of androgen activity. I still do not know this site.

FACTORS CONTROLLING GROWTH AND FUNCTION

Chairmen: Gregory Pincus and Vincent Hollander

GROWTH AND DEVELOPMENT OF THE HUMAN PROSTATE[1]

WILLIAM WALLACE SCOTT, Ph.D., M.D., The James Buchanan Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, Maryland

There are many aspects of the growth and development of the human prostate that we might discuss profitably. Because of the frequency of benign prostatic hyperplasia and prostatic cancer, I have chosen to discuss these growths because: 1) Both probably arise in prostatic tissues sensitive to circulating "androgens" and "estrogens" and hence may be responsive to endocrine manipulations; 2) the site of origin of each growth may tell us something of the "functional" capacity of the lobes, areas, or zones from which each arises.

Initially I wish to review several morphological studies related to these problems and later to call attention to the paucity of work in humans in which distinct chemical and functional differences between lobes of the prostate have been demonstrated.

ORIGIN OF THE HUMAN PROSTATE

Considerable controversy exists concerning the origin and development of the prostate gland of man, especially its division into various lobes, areas, or zones, the anatomic and functional differences of these areas, and the site of origin of disease processes of this gland.

Lowsley (*1*) reported that the glandular portion of the prostate arises from the epithelium of the narrow tubular structure of cloacal entoderm, the primitive posterior or prostatic urethra. Such glandular tissue becomes evident at about the 13th week of gestation when epithelial buds begin to extend posteriorly from the primitive urethra into surrounding mesenchyme. Later, five groups of epithelial buds are recognizable and termed as middle, right and left lateral, posterior, and anterior groups. The glands of the lateral group grow and eventually make up most of the substance of the adult prostate gland. The posterior and median groups develop to a lesser extent and the anterior group develops least of all.

According to Jores (*2*), in addition to these groups described, other glands develop from the posterior aspect of the urethra between the verumontanum and vesicle outlet, and these he termed the *suburethral* or *submucosal glands.*

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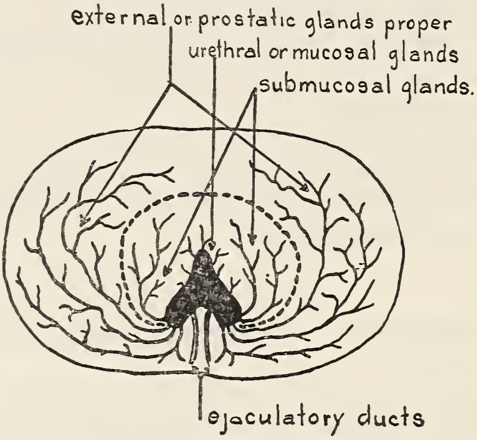
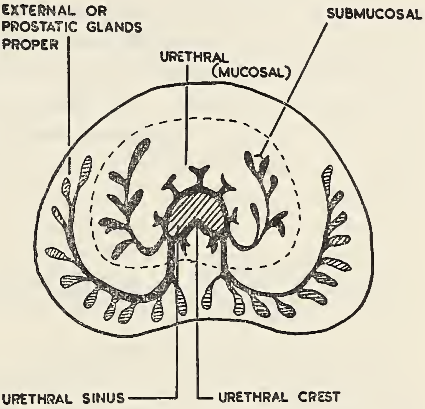
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962

The origin of the prostate was restudied by Loeschke (*3*) and Adrion (*4*), who divided the glands of the area into three categories according to their length (text-fig. 1): 1) The *mucosal* glands, which are relatively short and simple, surround the urethra laterally and anteriorly at the level of the verumontanum and posteriorly at the bladder neck; 2) the *submucosal glands,* which have longer ducts entering the urethra, are more branched; and 3) the *prostatic glands* proper, which have much longer ducts also entering the urethra on either side in the posterolateral sulci, show much more branching and lie outside the submucosal glands and mucosal glands. These authors called attention to "a definite fibrous capsule" separating the mucosal and submucosal glands ("inner glands") from the prostatic glands proper ("outer glands"). Jacoby (*5*), in a study of 200 autopsy specimens, confirmed the findings of Loeschke and Adrion, except that the capsule separating the inner and outer groups of glands was by no means a constant finding.


external or prostatic glands proper
urethral or mucosal glands
submucosal glands.
ejaculatory ducts

TEXT-FIGURE 1.—Diagram showing the anatomy of the normal prostate gland, according to Loeschke and Adrion. *Dotted line* indicates "a definite fibrous capsule" (*3, 4*). [From Young and Davis (*7*). Courtesy W. B. Saunders Co., Philadelphia, Penna.]

Recently, the problem has been restudied by Franks (*6*), and I quote several passages from his study: "Anatomically the prostate is generally divided into five lobes—posterior, middle, anterior and two lateral—related to the structures which pass through it. The posterior lies behind the urethra and deferential canal. The middle lobe lies between the urethra and the deferential canal and the anterior (lobe) in front of (*i.e.,* anterior to) the urethra. The lateral lobes lie on either side of the urethra." However, "On cross section of the gland there is thus no lobar pattern, but three concentric regions, of increasing size, from within outward can be recognized" (text-fig. 2). "The main mass is composed by long-branched 'external' glands—the prostatic glands proper—leading into ducts which curve backwards to open in the postero-lateral grooves. This thick outer layer invests a smaller mass of inner secreting elements and is separated from it by an indefinite capsule. The inner group consists of a large set of fairly long-branched submucosal units, the ducts of which open into the postero-lateral grooves, and a smaller mucosal group of short glands surrounding the urethra" (fig. 1).



Text-figure 2.—The distribution of the normal prostate glands (transverse section). Slightly modified from Grant, J. C. B.: *A Method of Anatomy.* [From Franks (*6*). Courtesy *Annals of the Royal College of Surgeons of England.*]

The early work of Loeschke and Adrion and the recent work of Franks essentially agree.

ORIGIN OF BENIGN PROSTATIC HYPERPLASIA

In 1926, Young and Davis (*7*) and, in 1954, Franks (*6*) reviewed the literature regarding the site of origin of benign prostatic hyperplasia. The observations by Morgagni (*8*), Jores (*2*), Motz and Perearneau (*9*), Tandler and Zuckerkandl (*10*), and Papin and Verliac (*11*) leave little doubt that benign hyperplasia arises from the mucosal or submucosal glands, *i.e.*, the "inner glands" referred to by Loeschke and Adrion. Figure 2 is taken from the studies by Jacoby (*5*) and shows small and early spheroids of hyperplasia just beneath the urethral mucosa, with ducts still communicating with the urethra.

Although there seems to be unanimity of opinion regarding the general site of origin of prostatic hyperplasia, considerable discussion continues as to whether the initial change takes place in the glandular epithelium or in the periglandular stroma near the urethra. Reischauer (*12*) claimed the initial change occurred in stroma through the development of fibromuscular nodules that, in turn, stimulated proliferation of the adjacent glandular epithelium. Whereas this theory has been supported by a number of workers, including Oberndorfer (*13*), Deming and Neumann (*14*), le Duc (*15*), and Moore (*16*), in a recent study Franks (*6*) states: "However, whether the change begins in the epithelium or in the stroma is still open to question."

ORIGIN OF PROSTATIC CANCER

The most extensive investigations into the site of origin and the nature of the tissue of origin are those of Geraghty and Boyd (*17*) in 1912, Moore (*18*) in 1935, Rich (*19*) in 1935, Kahler (*20*) in 1939, and Franks (*21*) in 1954.

Geraghty and Boyd found the initial lesion closely related to the tubules of the posterior lobe in 75 percent of cases. Moore reported that 73.5 percent arose in the posterior lobe—". . . that portion of the gland posterior and postero-lateral to the fan-shaped ducts which enter the sides of the urethra"—and Rich stated: ". . . it is perfectly clear that there is a decided tendency for the very early tumors to be situated either laterally or posteriorly in the tissue near the capsule of the gland." Kahler, in a study of 195 prostatic cancers, found that the number of cancers arising in the lateral lobes slightly exceeded the number arising in the posterior lobes. Distribution seemed to him to be roughly proportional to the cross-sectional area of the particular lobe. No cancer arose in the medial lobe.

Commenting on the nature of the tissue of origin, Moore stated: "Carcinoma of the prostate is intimately associated with senile atrophy and probably is derived from epithelial cells which have previously undergone atrophy." Similarly, Rich reported his series ". . . suggests that the non-hypertrophied senile tissue and the compressed atrophic glands outside the expanding nodules of hypertrophy are, perhaps, favorite sites of origin." These studies by Moore and Rich also imply that prostatic cancer arises from the outer prostate.

In 1954, Franks, basing his study of so-called "latent" prostatic cancer on 220 specimens, concluded that carcinoma of the prostate ". . . almost invariably begins in the outer glands, *i.e.*, the prostate proper" (figs. 3, 4, 5, and 6). "In only one case in the present series was there a small tumor nodule in the inner part of the prostate not in continuity with larger tumor masses in the prostate proper." Flocks (*22*) also emphasized the apparently different sites of origin of prostatic hyperplasia and cancer: "Benign prostatic hyperplasia consists in the growth of single or multiple adenomatous masses that compress the surrounding prostatic tissue. They start in the inner portion of the prostate, surround and distort the urethra, and compress the outer portion of the gland so that it forms the so-called false or surgical capsule. In contrast, carcinoma of the prostate rarely begins in this inner portion of the gland and rarely is totally confined to this region." One of the figures in Flocks' article (credited to Miguel Servet) shows a focus of cancer "in the midst of a large hyperplastic nodule" with no carcinoma in the posterior lamella. "However, further sections nearer the bladder neck revealed carcinoma in the posterior lamella also." This may have also been true in the single instance reported by Franks in which a cancer appeared to have arisen in the "inner" prostate.

Two additional morphological observations are worth presenting before this phase of the discussion is concluded.

It is not uncommon to find patients with a cancer within an adenoma that has been enucleated surgically on the basis of a clinical impression of benign prostatic hyperplasia. Such patients often do well and live for years. Unfortunately, no one has ever reported sectioning the remainder of the gland to see if there was residual cancer in the "outer" prostate. This should be done.

Finally, Dr. Robert Edelman, of our Department of Pathology, and one of our medical students, Mr. Martin Robson, recently observed what appeared to be complete infarction of the "outer" prostate in a patient who died of leukemia (figs. 7 and 8). The "inner" prostate seemed essentially normal. Such an observation is new to me and suggests a possible dual blood supply.

FUNCTIONAL AND CHEMICAL DIFFERENCES IN INNER AND OUTER ZONES OF THE HUMAN PROSTATE

In the animal, as has already been discussed and undoubtedly will be considered further as this Workshop continues, there are distinct functional and chemical variations between different lobes of the prostate. The question naturally has arisen as to whether such differences exist in the human being. In the rodent, in which the prostatic lobes are discrete, numerous investigators have shown that these lobes differ in histology, function, and concentrations of natural biochemical constituents, such as fructose and citric acid [Humphrey and Mann, 1948 (*23*), and Price, Mann, and Lutwak-Mann, 1949 (*24*)]; amino acids [Awapara, 1952 (*25*)]; acid and alkaline phosphatases [Fischer, Tikkala, and Mawson, 1955 (*26*)]; carbonic anhydrase [Mawson and Fischer, 1952 (*27*)]; and zinc [Mawson and Fischer, 1951 (*28*), and Gunn, Gould, and Anderson, 1962 (*29*)].

To my knowledge, Huggins and Webster (*30*) were the first to suggest a functional difference between the "posterior lobe" and the "anterior prostate" in man. They observed that, after administration of testosterone propionate, 10 mg daily for 21 days, followed by diethylstilbestrol, 80 mg intramuscularly in 59 days, to a 28-year-old male dying with an embryonal carcinoma of the testis, the acceptance of eosin stain differed in the anterior and posterior portions of the prostate (fig. 9). I quote: "In the present study, it was observed that the macrophage granules stain red with eosin (lipo-protein complex?). The presence of macrophages and this red-staining material were utilized in differentiation of the human prostate. . . . this red complex appeared only in association with the posterior lobe, being found in and around the epithelial cells in the lumen and in macrophages there. In a beautiful manner the posterior lobe could be differentiated by the rosy aura which this granular material cast about this region." Huggins and Webster concluded:

"Estrogen induces qualitative changes of degeneration to a greater extent in the anterior region of the human prostate than in the posterior lobe. The human prostate consists of two different physiologic units, the anterior prostate and the posterior lobe. . . . The posterior lobe which gives rise to cancer frequently in man has different characteristics than all of the prostatic cells anterior to it. The cells of the anterior prostate for an unknown reason commonly form spheroids in old men and rarely cancers."

Without disputing Huggins and Webster's observations, I believe the area below the heavy black line in figure 9 would correspond only *in part* to the "outer" prostate referred to by other investigators. Why, if a functionally distinct "outer" prostate exists, the anterolateral portion did not show this rosy aura to eosin stain remains unexplained.

A fairly exhaustive review of the literature fails to reveal any other studies that suggest chemical and functional differences in "inner" and "outer" zones or "anterior" and "posterior" lobes of the human prostate. For example, according to Gunn, Gould, and Anderson (*29*): "Investigators who have conducted zinc (*31–33*) and acid phosphatase (*32*) analyses on human prostate have found extreme variations in results even when samples were taken from the same lobe of the prostate. Kerr, Keresteci, and Mayoh (*33*) noted that the anatomic distribution of zinc in human prostate did not follow conventional anatomic planes, but, in general, was greater toward the apex of the gland." Of course, differences in results may be a reflection of variations in sampling of tissue, *e.g.*, the number of epithelial cells in the sample as compared to the amount of fibromuscular stroma.

FUNCTIONAL AND CHEMICAL DIFFERENCES IN PROSTATIC CANCER AND BENIGN NODULAR HYPERPLASIA

Obviously the greatest functional difference between prostatic cancer and hyperplasia is the capacity of prostatic cancer to invade beyond the prostate, to spread to distant sites via the lymphatics and the blood stream, and to kill. Yet each process seems somewhat dependent for growth on certain gonadal hormones. Thus, Huggins and his associates (*34, 35*) have shown that orchiectomy, or estrogen administration, or both profoundly influence the growth of prostatic cancer and to a lesser extent benign nodular hyperplasia. The dramatic clinical improvement in many patients with prostatic cancer after such therapy serves as a continuing stimulus to explore more fully the dependence (and independence) of the prostate and its outgrowths on hormones. To some of us, the possible dependence of benign hyperplastic prostatic tissue on hormones is equally challenging.

Investigators who have conducted histochemical studies on normal, hyperplastic, and cancerous prostatic tissue have found more profound histochemical changes in cancer than in hyperplasia. For example, Brandes and Bourne (*36*) in a summary of their findings stated: "The most striking changes in carcinoma were a reduction in the α-naphthyl esterase and periodic acid-Schiff reactions, an increase of fat, and a decrease and redistribution of acid phosphatase. In adenomatous prostates there was a moderate decrease in the α-naphthyl esterase reaction and a slight intensification of the succinic dehydrogenase and sulfide and disulfide reactions."

Andrews and Taylor (*37*), in metabolic studies on surviving human prostatic tissues, found that the Q_{O2} of human prostatic "adenoma" as measured by the Warburg technique varied between 0.65 and 5.67, with an average of 2.42. In two experiments the Q_{O2} of prostatic cancer was higher than in benign hyperplasia. However, "It was considered that the oxygen uptake of prostatic 'adenoma' depended on the number of epithelial cells in the 'adenoma' and on the secretory activity of the epithelial cells. It appeared probable that the higher value obtained with carcinoma was due to the larger number of epithelial cells." Here again, variations in results seem to have depended largely on differences in sampling.

Regarding manometric studies of respiration and glycolysis, I hope Dr. Horst Schirmer will comment, especially on his recent findings concerning the effect of acetylcholine on glycolysis.

Finally, in a recent electron microscopic study of the prostatic cancer cell, Takayasu and Yamaguchi (*38*) reported: ". . . that all prostatic cancer cells do not have characteristic features which make diagnosis of malignancy always possible. However, the electron microscopic features of many prostatic cancer cells present differences from those of epithelial cells of benign prostatic hypertrophy. The most remarkable difference exists in the nature of the mitochondria. Marked variation in size and number of the mitochondria is present in prostatic cancer cells, while the variation is uncommon in benign prostatic hypertrophy."

SUMMARY

Concepts have been presented regarding the origin of the human prostate and the zones of the prostate from which benign hyperplasia and cancer may arise. Except for evidence suggesting that different zones give rise to distinct disease processes, there is a paucity of data regarding distinct functional and chemical differences in zones of the normal human prostate as opposed to the situation in some animals, such as the rodent.

I believe that it may prove profitable to use the available techniques of electron microscopy, histochemistry, and metabolism to clarify the duality of the human prostate and the site of origin of its two major disease processes. Already, Dr. Brandes and I have begun such studies. Initially, we are directing our attention toward securing tissues, representative of specific zones or lobes of the prostate, and obtaining normal prostatic tissue in the fresh state. Also, some success has been achieved in the separation of epithelial components from fibromuscular stroma to minimize variations in results as a consequence of variations in sampling of the tissues used for any analysis or metabolic study.

If a distinct duality of the human prostate can be demonstrated, and in turn, this resembles the situation in the rodent, studies of the rodent may prove more useful toward a better understanding of the human prostate.

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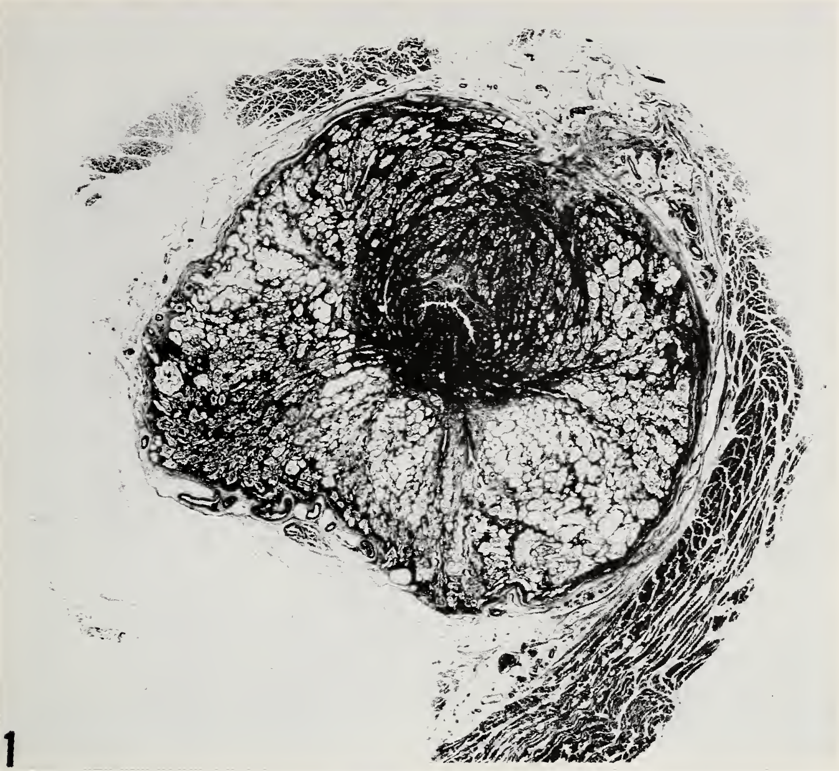
Plate 26

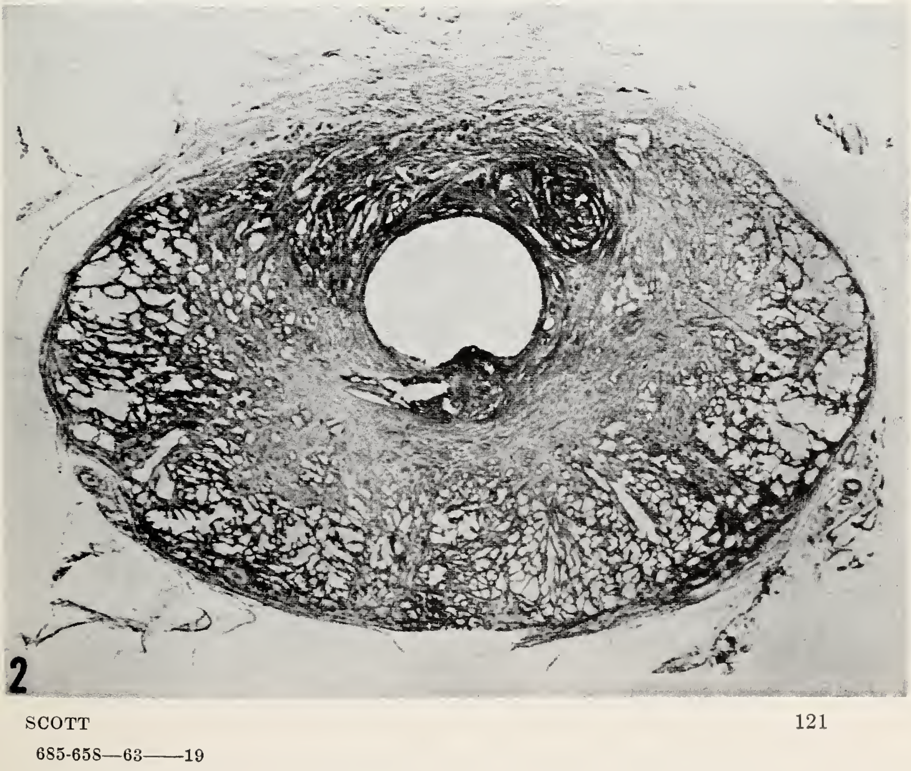
Figure 1.—Normal prostate, showing inner and outer gland groups (transverse section).

[Courtesy *Annals of the Royal College of Surgeons of England* (*6*).]

Figure 2.—Cross section of the prostate of a 62-year-old man, showing a very early stage of benign hyperplasia. *Note* 2 small spheroids of hyperplasia appear to arise in the inner zone while the rest of prostate is normal.

[Courtesy *Zeitschrift für Urologische Chirurgie* (*5*).]



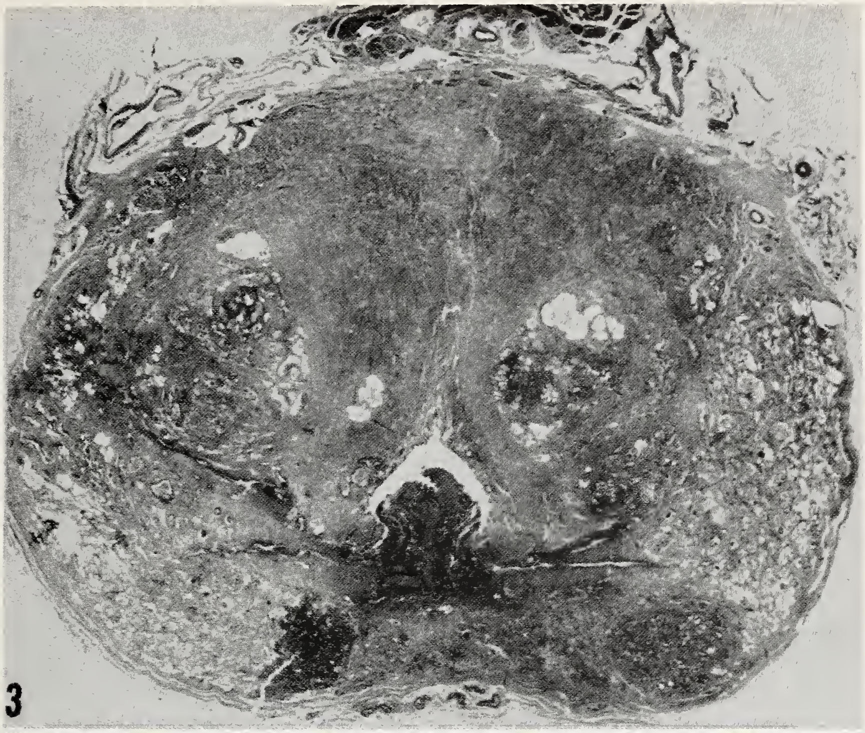


SCOTT

Plate 27

Figures 3 and 4.—Two prostates in transverse section, showing common site of origin of carcinoma in outer zone of prostate.

[Courtesy *Journal of Pathology and Bacteriology* (*21*).]



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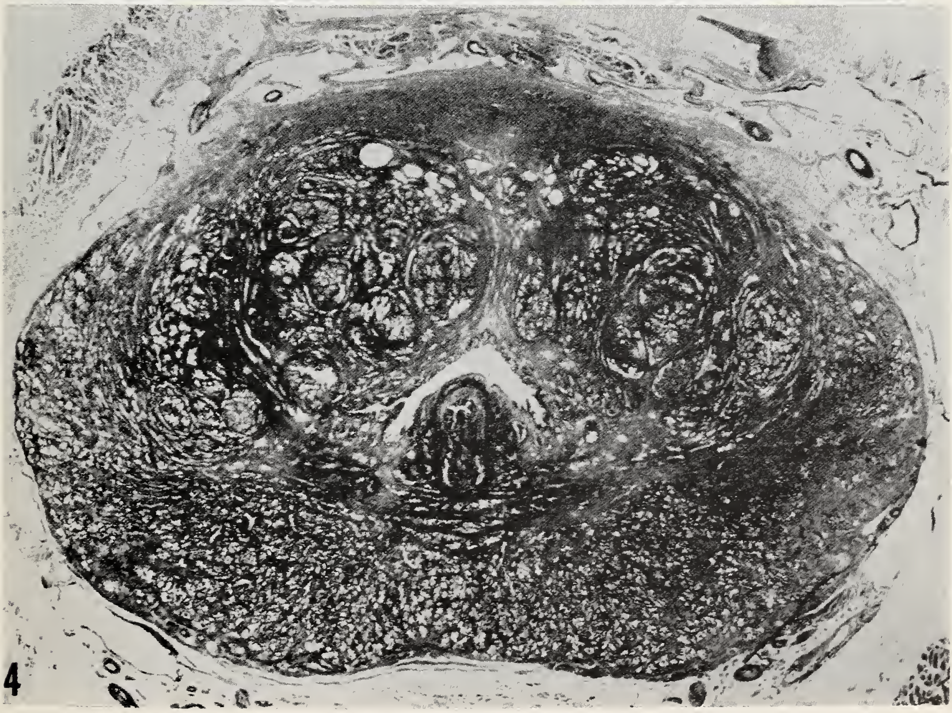

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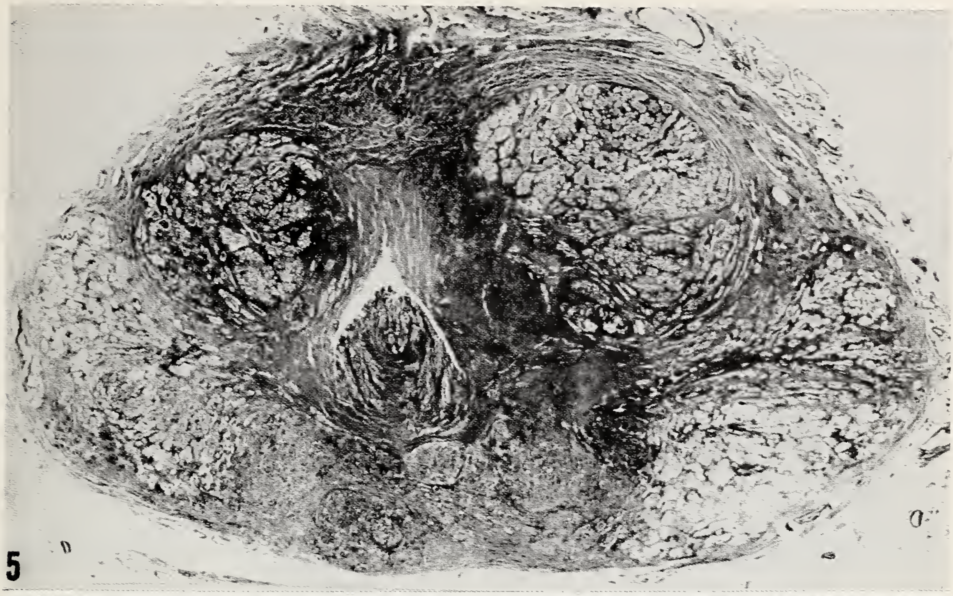
Plate 28

Figures 5 and 6.—Two prostates in transverse section, showing common site of origin of carcinomas in outer zone of prostate.

[Courtesy *Journal of Pathology and Bacteriology* (*21*).]



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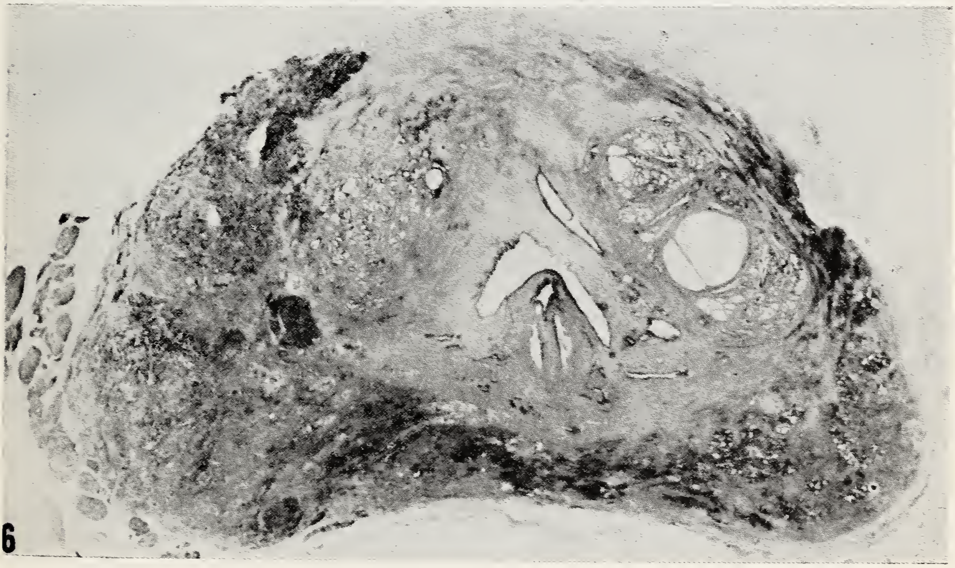

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Plate 29

Figures 7 and 8.—Gross and microscopic (× approximately 2½) cross sections of what appears complete infarction of outer zone of prostate, with preservation of inner zone.

[Courtesy Dr. Robert Edelman and Mr. Martin Robson.]

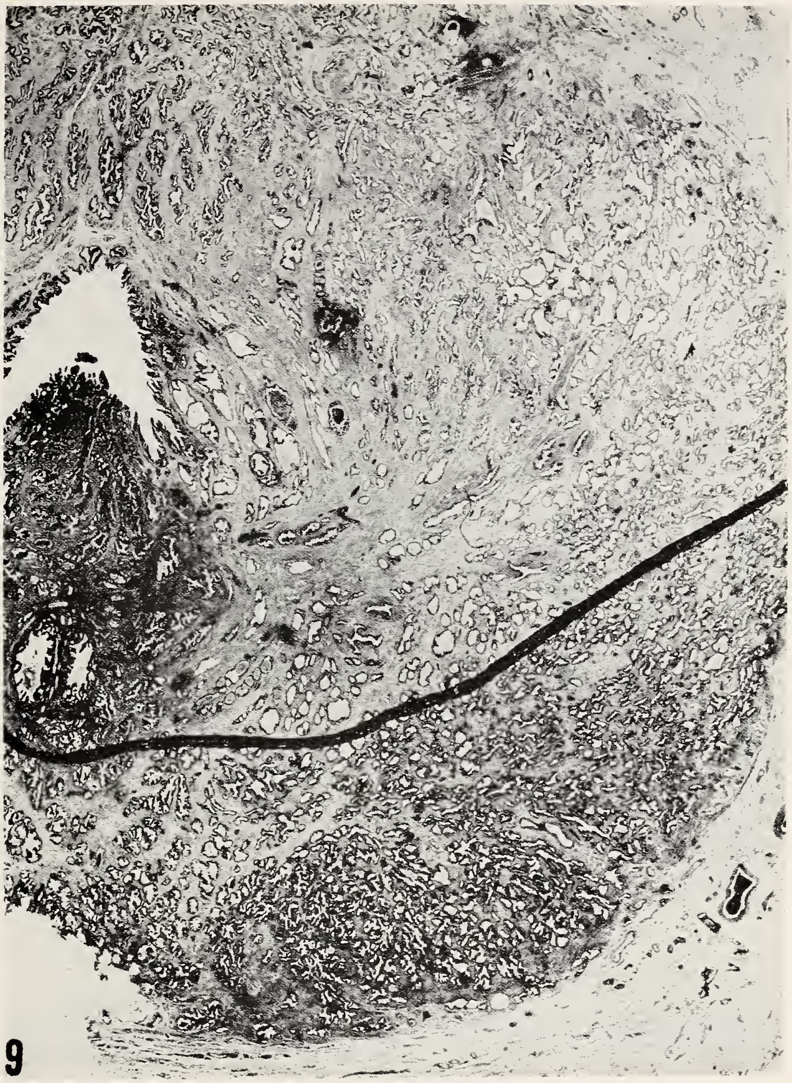
7

8

Plate 30

Figure 9.—Posterior lobe in a 28-year-old man. *Heavy black line* shows sharp separation between posterior lobe *below* and anterior prostate.

[Courtesy *Journal of Urology* (30).]



9

SCOTT

DISCUSSION

Dr. Price: Do you feel that the subcervical glands of Albarran are of importance in the development of benign nodular hyperplasia of the prostate?

Dr. Scott: I understand that at times Albarran's glands share in the development of the process, which clinically we call benign nodular hyperplasia, and that these glands are really submucosal glands (inner prostate) and not mucosal glands.

Dr. Mann: What are the present views on the significance of corpora amylacea in relation to the pathology of the human prostate?

Dr. Scott: I know what corpora amylacea look like under the microscope, but I do not know what their function is, if any.

Dr. Brendler: I have come across some work involving experimental tumors in rodents, where structures resembling corpora amylacea have been found in the dilated acini. Whether these are actually corpora amylacea I do not know.

Dr. Jensen: I understand that at one time there was a belief that the benign prostatic type of hyperplasia might be inhibited by androgen and that androgen was actually used for this purpose. What was the basis of this belief, and what is the status of it at present?

Dr. Scott: I believe that there has been no carefully controlled study on the hormonal control of benign nodular hyperplasia in which either androgens or estrogens were used. However, this may prove to be a fruitful field.

STUDIES IN PROSTATE CANCER BEFORE AND AFTER ORCHIECTOMY[1, 2]

T. F. GALLAGHER, WILLET F. WHITMORE, JR., BARNETT ZUMOFF,[3] and LEON HELLMAN,[3] Sloan-Kettering Institute for Cancer Research, New York, New York

SINCE the pioneer studies of Huggins and Hodges (*1*) there have been many attempts to relate steroid hormone metabolites with prostate cancer in humans. The reports of Birke, Franksson, and Plantin (*2*, *3*), Burt, Finney, and Scott (*4*), and Bulbrook, Franks, and Greenwood (*5*), among others, are noteworthy. The aim of the present study was twofold: to examine a group of patients with untreated prostate cancer before and after orchiectomy by isolation and measurement of individual metabolites of steroid hormones; to continue these studies of individual patients at appropriate intervals until death. Because the project has been long in progress it was not possible to apply today's advanced methodology in each instance. Nevertheless, there is sufficient information to evaluate the problem in the light of present knowledge, to suggest interpretations of findings, and to indicate where further exploration may yield meaningful results.

STEROID ISOLATION[4]

The methods employed for isolation, characterization, and quantitation of urinary steroid metabolites have been described, including the systems for chromatography (*6*). In all patients except DY, the values for dehydroisoandrosterone were so small that they would have no meaning. The clinical information on the patients studied is shown in table 1.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] This investigation was supported in part by a grant from the American Cancer Society, Inc., and research grant CY-3207 from the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] *Present address:* Montefiore Hospital, Bronx, N.Y.

[4] The following abbreviations and trivial names are employed:
Hydrocortisone: 11β,17,21-trihydroxy-Δ^4-pregnene-3,20-dione.
THF: 3α,11β,17,21-tetrahydroxypregnan-20-one.
THE: 3α,17,21-trihydroxypregnane-11,20-dione.
ATHF: 3α,11β,17,21-tetrahydroxyallopregnan-20-one.
A: androsterone; 3α-hydroxyandrostan-17-one.
E: etiocholanolone: 3α-hydroxyetiocholan-17-one.
D: dehydroisoandrosterone; 3β-hydroxy-Δ^5-androsten-17-one.
"Androgen" is used to indicate hormones that form androsterone, etiocholanolone, and dehydroisoandrosterone as metabolites.

Table 1.—Clinical description of patients with adenocarcinoma

Patient and age	Stage and grade	Quality of remission*
BT–52	D	G
CO–47	D	F
DY–45	C ii	G
FR–74	C	G
FA–73	C iv	G
HS–69	C iii	G
HL–55	C	G
KH–63	D	G
LI–69	D iii	P
LK–52	D iii	P
MA–72	D	F
PK–66	C ii	F
RT–50	D	G
SS–71	D? C ii	P
SO–68	D	G

Stages: A prostatic neoplasm too extensive for radical excision either because of local extent (stage C) or of metastases or both (stage D). Grades: the histological appearance of the neoplasm, increasing in malignancy to grade iv, which is a highly undifferentiated anaplastic tumor.

*G = good; F = fair; P = poor.

RESULTS AND DISCUSSION

The major hydrocortisone metabolites were studied in 4 patients immediately before and after orchiectomy, which caused remission of their disease (table 2). Three showed a significant fall in output of THF, THE, and ATHF following operation. The fourth patient showed a slight increase. After the operation all 4 patients excreted a normal amount of THF, though 2 had elevated THF levels before surgery. This is important because THF probably most accurately represents hydrocortisone, whereas the three metabolites reflect reactions in peripheral tissues that transform part of this hormone to biologically inactive cortisone. Three of the patients after operation exhibited a total output of the three

Table 2.—Hydrocortisone metabolites of patients with prostate cancer immediately before and from 7 to 10 days after orchiectomy; all patients had remission after operation

Patient and age	Period	Creatinine	THF	THE	ATHF	Total
			(mg/g creatinine)			
CO–47	Before	1. 2	3. 1	2. 4	0. 6	6. 1
	After	1. 3	1. 4	1. 4	0. 8	3. 6
SO–68	Before	1. 2	2. 8	2. 0	1. 1	5. 9
	After	1. 2	1. 7	1. 7	0. 6	4. 0
FR–74	Before	1. 6	1. 3	1. 5	0. 9	3. 7
	After	1. 7	1. 2	0. 8	0. 5	2. 5
HS–69	Before	1. 2	1. 2	1. 7	0. 6	3. 5
	After	1. 4	1. 3	2. 3	0. 4	4. 0
Mean normal values			1. 3	1. 8	0. 6	3. 7

metabolites at the normal level, and the fourth (FR) showed a subnormal total output in the presence of a normal amount of THF.

These findings, we believe, are consistent with the interpretation that most of these patients were in a state of acute alarm and mental stress prior to operation—they had very recently learned (*a*) they had cancer and (*b*) they were to lose their testicles. That in 3 patients the urinary levels of the three hydrocortisone metabolites reverted to normal or subnormal following their operations might plausibly be ascribed to relief of pain and lessening of anxiety about an accomplished fact. The fourth subject (HS), who showed no change, was greatly alarmed before and since his operation despite remission of his disease. Price, Thaler, and Mason (*7*) have reported elevated plasma 17-hydroxycorticosteroid levels in patients before thoracic and cardiac surgery and interpreted these as indicative of the emotional involvement of the patient.

The androgen metabolites were examined immediately before and very shortly after orchiectomy in 13 men (table 3). Every patient, without exception, exhibited a decrease in combined output of androsterone and etiocholanolone after orchiectomy. In some patients the change was small and in others relatively great. The results were strictly comparable with those reported by Birke *et al.* (*2*, *3*) and the series of Burt, Finney, and Scott (*4*).

TABLE 3.—Androgen metabolites of patients with prostate cancer immediately before, and from 4 to 40 days after, orchiectomy

		A		E		Total	
		(mg/g creatinine)					
Patient and age	Creatinine	Before	After	Before	After	Before	After
PK–66	1. 2	0. 6	0. 3	1. 6	0. 9	2. 2	1. 2
FA–73	1. 1	1. 0	0. 5	2. 0	0. 9	3. 0	1. 4
DY–45	1. 4	3. 4	1. 8	2. 4	1. 6	5. 8	3. 4
HS–69	1. 1; 1. 4	1. 8	0. 6	1. 9	1. 2	3. 7	1. 8
CO–47	1. 2; 1. 3	0. 8	0. 6	1. 9	1. 2	2. 7	1. 8
FR–74	1. 6; 1. 7	0. 9	0. 3	0. 8	0. 5	1. 7	0. 8
SO–68	1. 2	0. 8	0. 6	2. 3	2. 0	3. 1	2. 6
MA–72	0. 8; 1. 0	0. 5	0. 4	1. 2	0. 9	1. 7	1. 3
KH–63	1. 0	1. 5	0. 4	1. 9	0. 6	3. 3	1. 0
HL–55	1. 2; 1. 3	0. 9	0. 4	2. 0	1. 0	2. 9	1. 4
SS–71	1. 1; 0. 9	1. 2	0. 4	1. 8	0. 6	3. 0	1. 0
LI–69	1. 0; 1. 1	0. 6	0. 5	2. 4	2. 1	3. 0	2. 6
LK–52	1. 6; 1. 2	0. 9	0. 4	1. 3	0. 7	2. 2	1. 1

In this series of patients there was no evident correlation between the level of urinary androgen metabolites before orchiectomy and the occurrence or lack of remission. One patient (DY), with a long remission, had a high initial androgen excretion, while PK, with a remission of comparable length, had a low initial value. One patient with a high androgen excretion level (LI) had no remission, while another (MA) with a much lower initial level benefited from the operation. There was, further, no evident correlation between the fall in androgen excretion after orchiectomy and remission or failure to improve. Three patients failed to have a remission of their disease; 2 of these exhibited a decrease in androgen approximately the equivalent of the mean of the whole series but the third patient showed a minimal drop in androgen metabolites. Of the 2 patients with the longest remission, 1 exhibited a substantial fall in androgens following operation, while the other showed a small decrease.

For the reasons that have been advanced, these differences before and after orchiectomy are probably exaggerated. There is considerable individual variation in the adrenal androgen response to stimulation. Moreover, it is difficult in retrospect to evaluate the impact of the psychological factors in each individual before and after operation. Predictably, these would vary widely. For these reasons then, to correlate urinary androgen metabolites before and after orchiectomy with the clinical response of the patient with prostatic cancer is unjustifiable because of the complexity of all the operative factors.

The androgen metabolites of 8 patients were examined serially for most of their lifetime after castration. Five of these subjects never showed any increase in output of androgen metabolites at any time after orchiectomy. The length of remission in these 5 patients ranged from 8 to 21 months. Three other patients with remissions from 12 to 46 months had an increased excretion of androgen metabolites in one of the periods examined. The detailed studies of these 3 patients are recorded in tables 4, 5, and 6.

One patient (DY) had an increased output of androgen metabolites (including dehydroisoandrosterone) 14 months after orchiectomy. This was some time before his relapse and careful examination of the record failed to reveal any circumstance to which this change could be ascribed. The same man had a low androgen output 22 months after orchiectomy. The only pertinent fact elicited at that time was that he had been "on vacation and drinking heavily." A smaller rise 54 months after orchiectomy was ascribed to the stress of an indwelling catheter that bothered the patient greatly.

Table 4.—Androgen metabolites of patient DY–45 at intervals after orchiectomy

Months	Creatinine	A	E (mg/g creatinine)	D	Total
"Control"	1. 4	3. 4	2. 4	3. 9	9. 7
1	1. 4	1. 8	1. 6	2. 2	5. 6
5	1. 4	1. 5	2. 8	2. 1	6. 4
9	1. 3	1. 3	2. 5	*	
14	1. 2	1. 7	3. 8	3. 1	8. 6
16	1. 3	1. 3	3. 3	1. 5	6. 1
22	1. 4	0. 7	1. 8	0. 9	3. 4
30	1. 4	1. 0	2. 6	1. 5	5. 1
34	1. 3	1. 5	3. 1	2. 3	6. 9
40	1. 4	1. 3	2. 9	1. 3	5. 5
44	1. 3	1. 6	2. 4	1. 5	5. 5
46	1. 3	1. 5	1. 7	1. 7	4. 9
54	1. 4	1. 6	2. 4	2. 0	6. 0
62†	1. 0	0. 5	1. 4	0. 6	2. 5

*Not measured.
†On estrogen.

TABLE 5.—Androgen metabolites of patient PK–66 at intervals after orchiectomy

Months	Creatinine	A	E	Total
		(mg/g creatinine)		
"Control"	1.1	0.6	1.6	2.2
10 days	1.2	0.3	0.9	1.2
1	1.2	0.2	0.6	0.8
9*	1.1	0.2	0.3	0.5
17*	1.2	0.2	0.3	0.5
25	1.3	0.3	0.6	0.9
29	1.2	0.3	1.4	1.7
32	1.0	0.2	0.2	0.4
54†	0.3	0.3	0.6	0.9

*Received estinyl during these studies.
†Radiation treatment during this study.

TABLE 6.—Androgen metabolites of patient KH–63 at intervals after orchiectomy

Months	Creatinine	A	E	Total
		(mg/g creatinine)		
"Control"	1.0	1.5	1.9	3.4
1	1.0	0.4	0.6	1.0
1*	1.3	0.4	0.5	0.9
1	1.3	0.9	1.0	1.9
12	1.2	0.6	2.3	2.9
15	1.0	0.6	1.4	2.0

*Received human growth hormone during this study; the subsequent study was immediately thereafter.

The second patient (PK) with a "secondary rise," which was confined to etiocholanolone, showed this change 29 months after orchiectomy. The patient at that time was concerned about the prolapse of an ileostomy, and the existence of an alarm reaction during the study period must be seriously entertained. The other patient (KH) showed an increase of androgen metabolites, again confined to etiocholanolone, about the time he relapsed 12 months after orchiectomy and when he was clearly aware of his clinical status.

It is evident from these results that either a minority of patients show the phenomenon of a "secondary rise" or that the patients who failed to show the change were not examined at the appropriate time. Clearly, either alternative presents a problem. In our opinion, if such a "secondary increase" of androgen metabolites does happen it can be ascribed to the patient's reaction to his own physical condition, *i.e.*, it is the equivalent of his alarm and stress prior to orchiectomy. It seems to us less than likely that any such increase could be a causative factor in the relapse. Most important is the fact that the adrenal "androgen" is not testosterone, though it yields some of the same metabolites. Moreover, at least in patient DY, the production of adrenal dehydroisoandrosterone was at a high level for any man with or without prostate cancer. This was compatible with an extended remission; a sharp lowering of androgen metabolites induced by estrogen administration after relapse was not accompanied by clinical improvement. It is our view that the amelioration of prostatic cancer induced by orchiectomy is caused by the removal of a testicular hormone and that subsequent changes in the adrenal secretions *reflect* alterations in the patient's status but are not causative of these.

CONCLUSIONS

The changes in steroid hormone metabolites after orchiectomy for prostatic cancer are a measure of a complex of reactions in the patient and not simply loss of testicular androgen. The factors of anxiety, pain, and stress contribute importantly to the metabolite pattern, if these are not indeed the major determinants. It is concluded that other means than urinary metabolite levels must be employed to evaluate the endocrine status of these patients and their response to treatment.

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DISCUSSION

Dr. Dorfman: Working on a similar problem with Dr. Strauss of Boston University, we demonstrated that ethynyl estradiol administered to men produces a profound lowering of plasma testosterone. The change is from about 0.6 μg per 100 ml of plasma to a value of less than 0.05 μg.

Dr. Fraumeni: I wonder, Dr. Gallagher, if an alternative interpretation, other than the "stress" hypothesis, might be invoked to explain some of the cases of prostatic cancer that were associated with elevated urinary corticosteroid metabolites. It is known that clinical, chemical, and pathological signs of adrenal hyperplasia may occur secondary to carcinomas of various organs. Anaplastic or oat cell carcinoma of the lung, thymomas, and pancreatic neoplasms head the list of those tumors, but prostatic cancer has also been reported (*1*, *2*). Studies by Holub and Katz (*3*) and by Meador and associates (*4*) have revealed that the nonendocrine tumor tissue in these cases may contain large quantities of a corticotropin-like substance. This is accompanied by subnormal pituitary content of ACTH, high plasma ACTH levels, and diffuse elevation of hydrocortisone and androgen metabolites in the urine. It is conceivable that autonomous secretion of an ACTH-like compound by prostatic tumors could produce some biochemical indexes of adrenal hyperfunction without signs of frank Cushing's syndrome, and that any successful therapy directed toward the cancer would inhibit production of this compound by the tumor and therefore depress steroid biosynthesis.

Dr. Gallagher: The prostatic tumor was still present after orchiectomy. It is not possible to rule out the factor you have suggested but surely there must be a considerable stress as well.

Dr. Fraumeni: Orchiectomy could result in shrinkage of tumor tissue, or cessation of growth, thereby suppressing this mechanism for hypercorticism.

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Dr. Scott: Was ACTH given to any of your patients in whom you measured urinary androsterone and etiocholanolone levels both before and after castration?

Dr. Gallagher: No.

Dr. Scott: In our experience, the administration of ACTH to a patient with prostatic cancer evokes a greater response in terms of urinary androsterone and etiocholanolone excretion after castration than before.

SOME ASPECTS OF RELATION OF STRUCTURE OF STEROIDS TO THEIR PROSTATE-STIMULATING EFFECTS[1]

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G. D. Searle & Co., Chicago, Illinois

In approaching a discussion of chemical structure in relation to biological activity it is necessary first to decide what endpoints we might select to assess activity. For some 10 years we have been conducting androgen assays in male rats, using the method of Eisenberg and Gordan (*1*). Increase in body weight and the final weights of the levator ani muscle and the seminal vesicles and ventral prostate glands have been used as measures of the effect. Testosterone, androsterone, and other common androgens increase the value of all of these parameters. However, not all compounds increase each parameter to the same extent. The anabolic steroids, such as 17-ethyl-19-nortestosterone, are effective in increasing body weight and levator ani muscle weight, but have much less effect on the seminal vesicle and ventral prostate (*2*). Thus, we can make some separation between anabolic steroids that are also potent androgens, such as testosterone, and those which are relatively devoid of androgenic activity (*2–4*).

Our separation of endpoints must go much further. It has long been known that estrogens will cause some stimulation of the seminal vesicle in castrated rats without significantly affecting the prostate weight (*5*). Saunders (*6*) presented data showing that as the dose of testosterone propionate was increased from 0.05 to 1.0 mg the seminal vesicles and ventral prostate weights were similarly enhanced, the latter reaching values around 125 mg. On the other hand, similar doses of estrone enhanced seminal vesicle weights to a maximum of about 30 mg but failed to alter prostatic weight. The increase in seminal vesicle weight, which was observed in the castrate after estrogen administration, has been shown to be due to increase in supporting structures rather than secretory epithelium (*5*).

In the intact adult animal, estrogens caused inhibition of pituitary gonadotropins with resultant testicular inhibition and an atrophy of the secondary sex organs (*7*). Similar effects were obtained by androgen administration, but here the exogenous androgen may compensate for the loss of endogenous androgen (*7*). Using 17α-methyl-19-nortestosterone, McGinty and Djerassi (*8*) showed that a daily dose of 0.5 mg caused marked inhibition of testes, seminal vesicles, and ventral prostates but, at a dose of 8 mg, the accessories were nearly twice the weights observed in intact animals. It should be possible in the intact male to obtain first testicular inhibition and then differential stimulation of the seminal vesicles or ventral prostate by the choice of an appropriate steroid.

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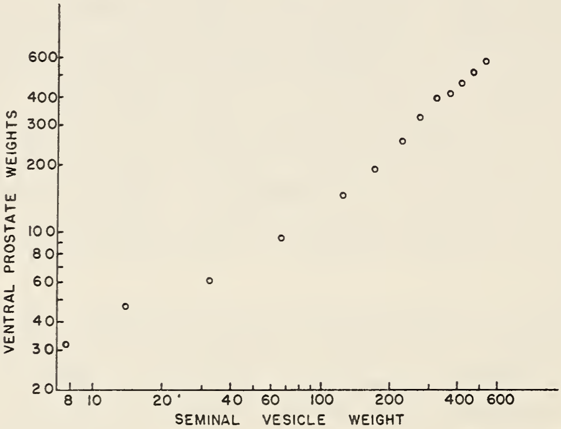
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] I wish to thank Dr. E. F. Nutting for some of the biological data included in this report, Drs. F. B. Colton, R. Dodson, W. M. Hoehn, R. C. Tweit, W. Johns, R. E. Counsell, and P. Klimstra, and Mr. L. N. Nysted and R. Ray who synthesized many of the compounds discussed here.

It would seem desirable first to study the relationship between these glands in intact, untreated males.

RELATION BETWEEN SEMINAL VESICLE AND VENTRAL PROSTATE WEIGHTS IN UNTREATED MALE RATS

Data were collected from 500 intact male rats of various ages. These had served as controls in various studies over a period of years. The data are grouped and plotted in text-figure 1.



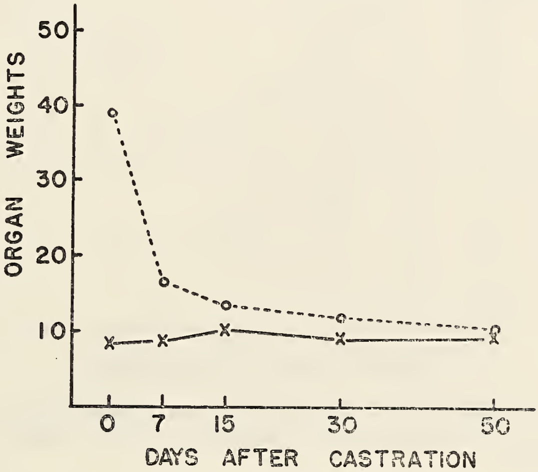
Text-figure 1.—Relation between ventral prostate and seminal vesicle weights (mg) in intact growing and mature rats. Grouping was made on the basis of seminal vesicle weights; 500 rats included in this figure.

The data were first grouped according to seminal vesicle weights and then the average values for the seminal vesicles and ventral prostates were calculated. Both ordinate and abscissa are on a log scale.

We note a break in this curve. The lowest values shown (48 rats) are at a seminal vesicle weight of 7.68 mg (all values were below 10 mg). The average prostate weight for this group of weanlings was 32.27 mg. The slope of this lower portion of the curve is shallow indicating a relatively faster growth of the seminal vesicle than of the ventral prostate until both glands approach a value of approximately 80 to 100 mg, after which growth of both glands proceeds at about the same rate.

EFFECT OF CASTRATION ON THE PROSTATE AND SEMINAL VESICLE

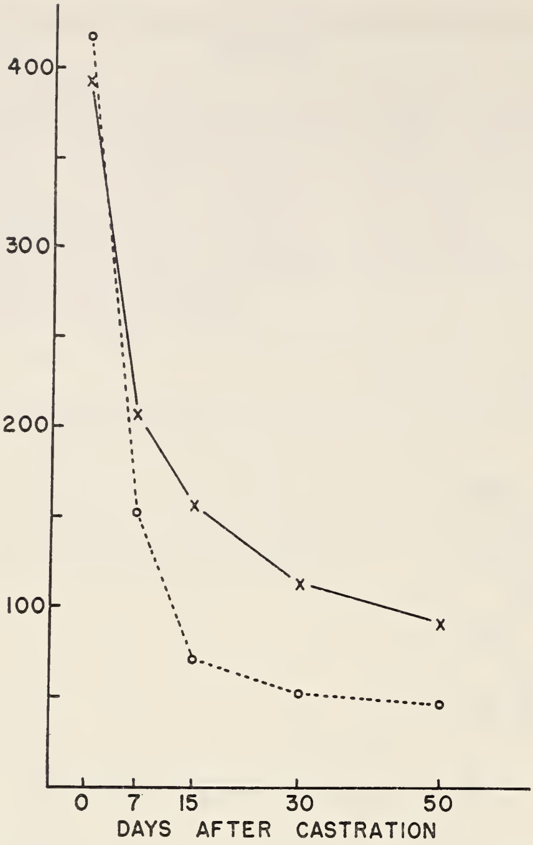
It seems worthwhile to consider the effect of castration on the weights of these accessories. The data shown in text-figures 2 and 3 are selected from data of Saunders (*9*). Text-figure 2 shows the decrease in these organs at various times after castration, performed in rats weighing 50 to 75 g. It is evident that the testes of these immature animals are secreting some hormone which promotes prostatic growth, but is insufficient in amount, or quality, to stimulate growth of the seminal vesicle. Thus, in these immature animals the prostate weight is about 4 times that of the seminal vesicles in the intact male but the 2 organs regress to the same weight after castration.



TEXT-FIGURE 2.—Change in the weights (mg) of the seminal vesicles (X) and ventral prostates (O) at various periods after castration. Rats weighed 50 to 75 g at time of castration.

These data constituted an important consideration in our choice of the type of rat used in our androgen assays. If the immature rat is used immediately after castration, as in the Hershberger modification (*4*), there is a marked regression in prostatic weight in the untreated control during the 7-day assay period. But, if one waits 2 or 3 weeks after castration before initiating treatment, the weight of this gland will have regressed to a more stable value. In the former case, a compound that reduces the rate of regression would be classed as active, while in the latter case an active compound must be capable of restoring glandular growth.

In the intact adult rat (text-fig. 3), the prostate and seminal vesicle weights are more nearly equal. After castration the atrophy of the



Text-figure 3.—Change in the weights (mg) of the seminal vesicles (X) and ventral prostates (O) at various periods after castration. Rats weighed 350 to 400 g at time of castration.

prostate is much more rapid and more complete than that of the seminal vesicle. Here again, the testes have a much more marked effect on the prostate than on seminal vesicle. Thus, the changes in prostate weight might be a more sensitive indicator of androgenicity than are the changes in seminal vesicle weights.

RELATION BETWEEN SEMINAL VESICLE AND PROSTATE IN STEROID-TREATED CASTRATED RATS [3]

Male rats were castrated at 23 days of age and steroid treatment was begun 18 days later. The total dose of steroid, dissolved in corn oil, was divided into 7 equal portions, one seventh being administered daily, subcutaneously, on successive days. On the day after the last injection the animals were killed, and the accessory organs dissected out and weighed.

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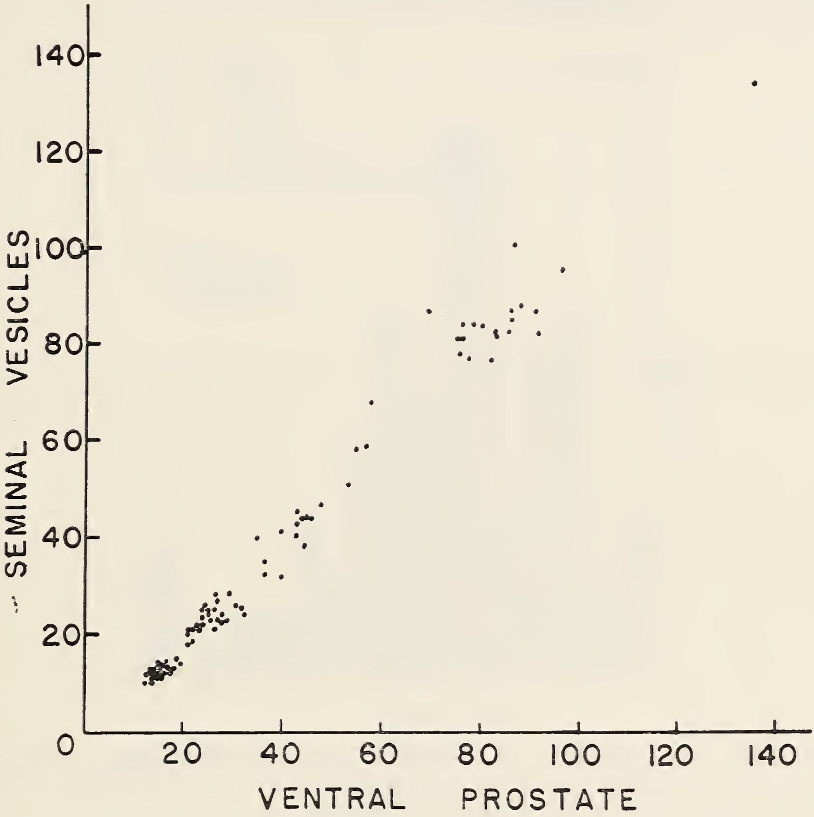
[3] Read in part at the International Congress on Hormonal Steroids. Milano, Italy, May 14–19, 1962.

Testosterone propionate was used as a standard. The results are depicted in text-figure 4. In this series the ratio of ventral prostate to seminal vesicle remained approximately unity whether the absolute weights were near the castrate level of 10 mg or increased to values of 100 mg or more.



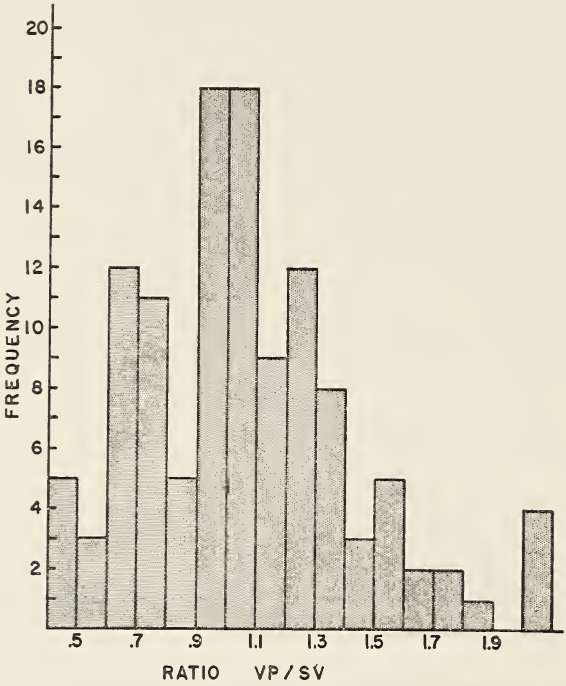
Text-figure 4.—Relation between seminal vesicle and ventral prostate gland weights (mg) in immature castrated rats treated with various doses of testosterone propionate. Each point shows the average value for a group of 8 rats: 93 treated groups are represented.

The ratio of ventral prostate weight to seminal vesicle weight was chosen as an endpoint for the comparison of a large series of steroids. We arbitrarily chose only those steroids that produced seminal vesicle or ventral prostate weights of at least 15 mg, when a total dose of 5 mg of the steroid per rat was used. There were some 120 steroids studied that met these qualifications.

The resultant ratio of ventral prostate to seminal vesicle, calculated from the uncorrected gland weights (VP/SV), varied from 0.39 to 3.82.

The distribution of the values of the VP/SV ratio, without regard to structure or potency, is shown in text-figure 5.

All the values for testosterone propionate fell within the range of 0.8 to 1.5 and 87 percent were between 0.9 and 1.3. In text-figure 5 there were 31 compounds with ratios lower than the minimum obtained with testosterone propionate and 14 with values above the maximum obtained with this standard. Thus a comparison of structures and the VP/SV ratio seemed justified.

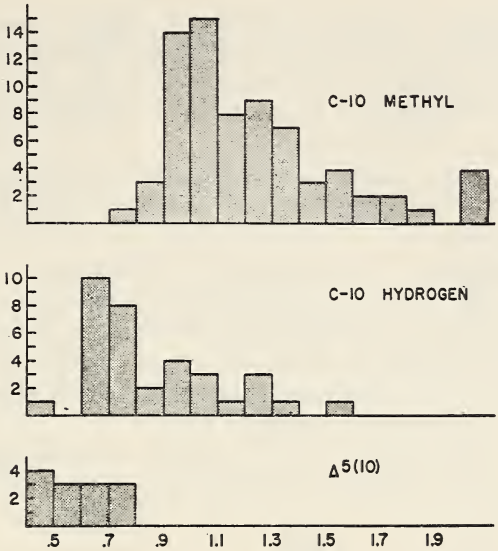


Text-figure 5.—Distribution of the ratio, ventral prostate/seminal vesicle, for groups of 8 castrated rats each receiving a total dose of 5 mg of steroid. The ratios obtained with 118 different steroids are shown.

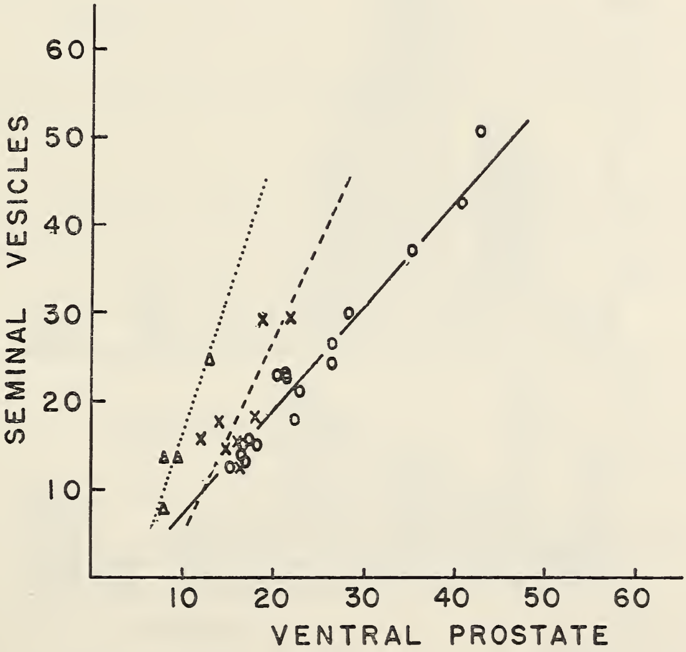
We first considered the effect of the substituent at C-10. The distribution of the VP/SV ratios is shown in text-figure 6.

There were 73 compounds that had a methyl group at C-10. The median value for the VP/SV ratio for these was 1.1. When this methyl group was replaced by hydrogen, as in the 19-norsteroids, the median value was 0.7 for 34 compounds. Only 13 androgens had a C-5(10) double bond. All of these produced low ratios, the median being 0.5. The shift, C-10 methyl → C-10 hydrogen → C-5(10) double bond seems to favor differentially the stimulation of seminal vesicle weight rather than prostate weight.

This relationship was studied further in the series: testosterone, 19-nortestosterone, and $\Delta^{5(10)}$-estren-17-ol-3-one. A range of doses was used and the resultant organ weights are shown in text-figure 7. It is evident that



TEXT-FIGURE 6.—Effect of the presence or absence of a methyl group on C-10, or a 5(10) unsaturation, on the value of the VP/SV ratio (ordinate: number of compounds).



TEXT-FIGURE 7.—Relation between seminal vesicle and ventral prostate weights (mg) in rats treated with various doses of $\Delta^{5(10)}$-estren-17-ol-3-one, (Δ), 19-nortestosterone (X), or testosterone (O). Each point is the average value for a group of 8 rats and the curves were calculated from the data.

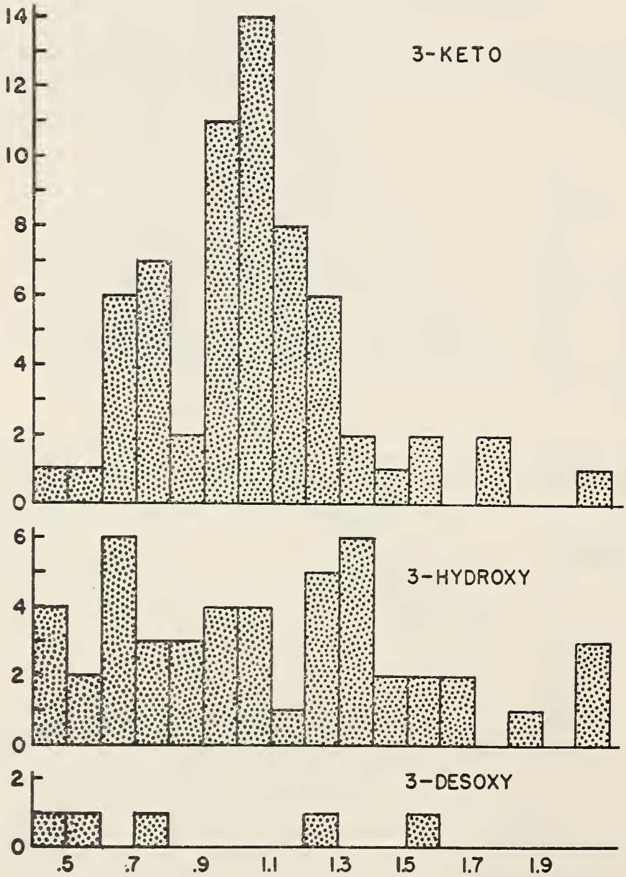
with testosterone a moderate increase in seminal vesicle weight was accompanied by a comparable increase in ventral prostate weight. This was not the case with the $\Delta^{5(10)}$ estrenolone. For this series, therefore, the predicted divergence of prostate and seminal vesicle weights was observed.

With each of these compounds, as with testosterone propionate (text-fig. 4), the VP/SV ratio was relatively constant, regardless of the magnitude of the response.

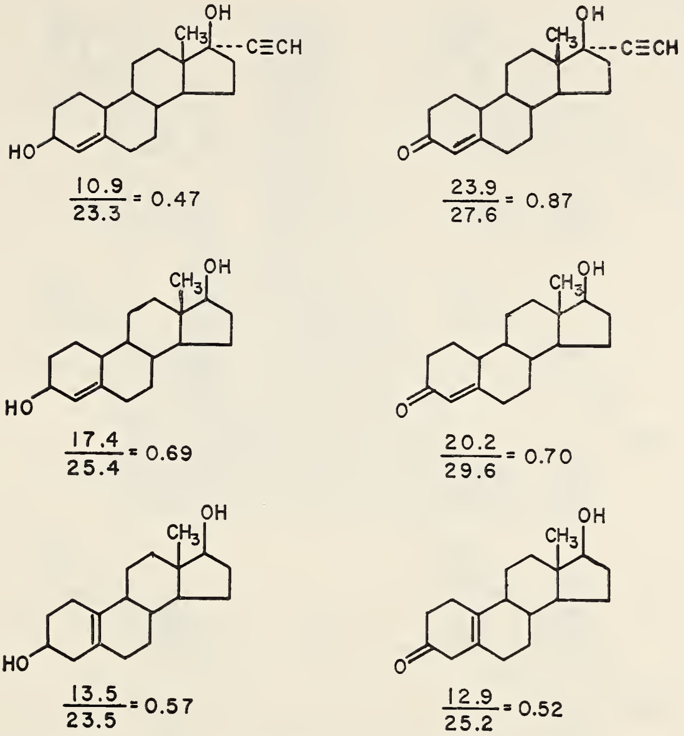
We next considered the importance of the oxygen function at C-3. The data on 117 steroids are shown in text-figure 8. There was no apparent difference in the distribution whether the C-3 atom had a keto group, a hydroxyl group, or lacked an oxygen function.

To check this point further, we selected a number of steroids differing only by a hydroxyl or a keto group on C-3.

Text-figure 9 shows 3 of these pairs. We see that ethynyl-19-nortestosterone has a relatively much greater effect on the prostate than



Text-figure 8.—Effect of an oxygen function on C-3 on the distribution of the values of the VP/SV ratio (ordinate: number of compounds).



$\frac{10.9}{23.3} = 0.47$

$\frac{23.9}{27.6} = 0.87$

$\frac{17.4}{25.4} = 0.69$

$\frac{20.2}{29.6} = 0.70$

$\frac{13.5}{23.5} = 0.57$

$\frac{12.9}{25.2} = 0.52$

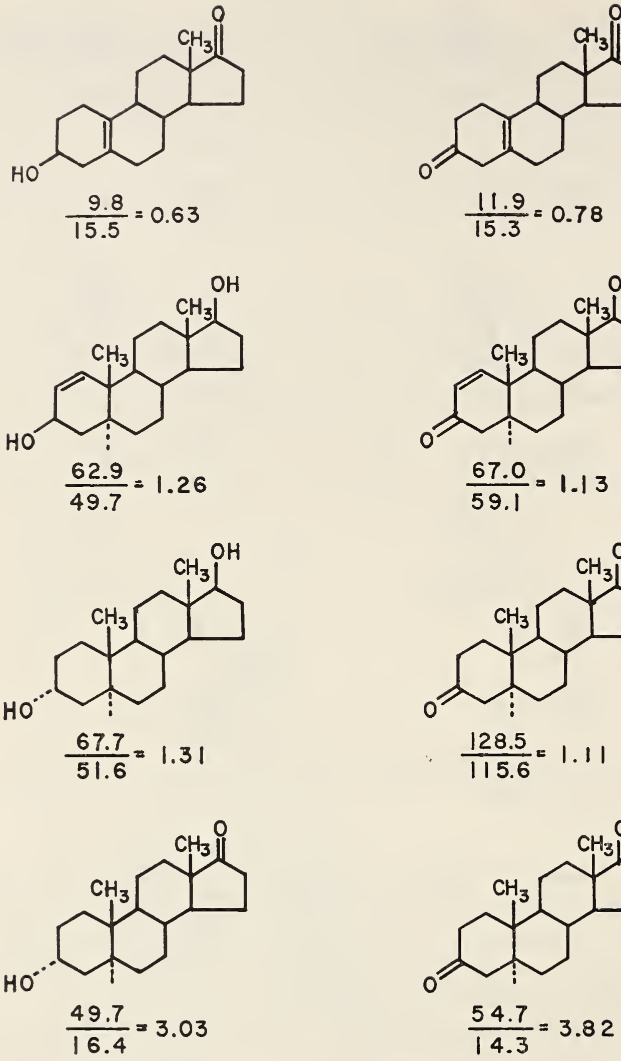
TEXT-FIGURE 9.—Structural formulas and VP/SV ratios of several 3-hydroxy steroids and their 3-keto analogue.

does its 3-hydroxy analogue. Without the 17-ethynyl group, 19-nortestosterone differs little from the diol. The same is true when the double bond is shifted to the 5(10) position. The seminal vesicle response to the 5 mg dose was similar with all 6 of these compounds but the prostatic response was much more variable.

The $\Delta^{5(10)}$-17-keto compounds shown in text-figure 10 had only minimal androgenic activity. The Δ^1 analogue of testosterone and its corresponding diol were much more potent androgens and stimulated both prostates and seminal vesicles. Dihydrotestosterone and its 3-hydroxy analogue are even more potent androgens. Androstanedione and androsterone both produce primarily prostate stimulation.

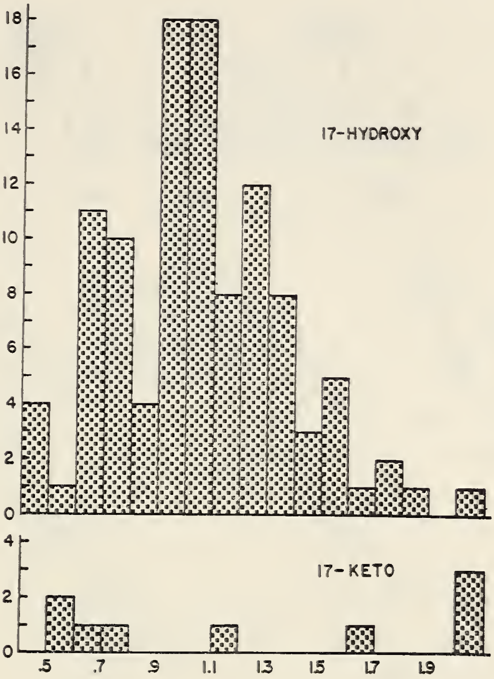
It would thus appear that the presence of either a 3-hydroxy or a 3-keto grouping is consistent with both prostate and seminal vesicle-stimulating activity.

The effect of the oxygen function at C-17 is depicted in text-figure 11. Unfortunately, the number of ketones available was relatively small. We note, however, that most of the ketones were found at the two ends of the scale. This would appear to be dependent on factors other than the C-17 oxygen.



Text-figure 10.—Structural formulas of some 3-hydroxy steroids and their 3-keto analogues, together with their VP/SV ratios.

Further scrutiny of the structures revealed that a number of compounds tested differed only in the substituents on C-17. These are compared in text-figure 12. It is evident the 17-keto compound exhibited a higher ratio than did the corresponding 17-hydroxy compound in every case studied. This effect of the 17-oxygen was also illustrated in text-figure 10 where androsterone (3.03) and androstanediol (1.31) were compared. Additional examples will be shown later (table 1). However, the 17-hydroxy compounds were more potent in nearly every case studied. The picture is further complicated by the fact that estrone, with a 17-ketone group, stimulates seminal vesicle growth rather than ventral prostate.



TEXT-FIGURE 11.—Distribution of the VP/SV ratios with compounds having a hydroxyl or keto group on C-17 (ordinate: number of compounds).

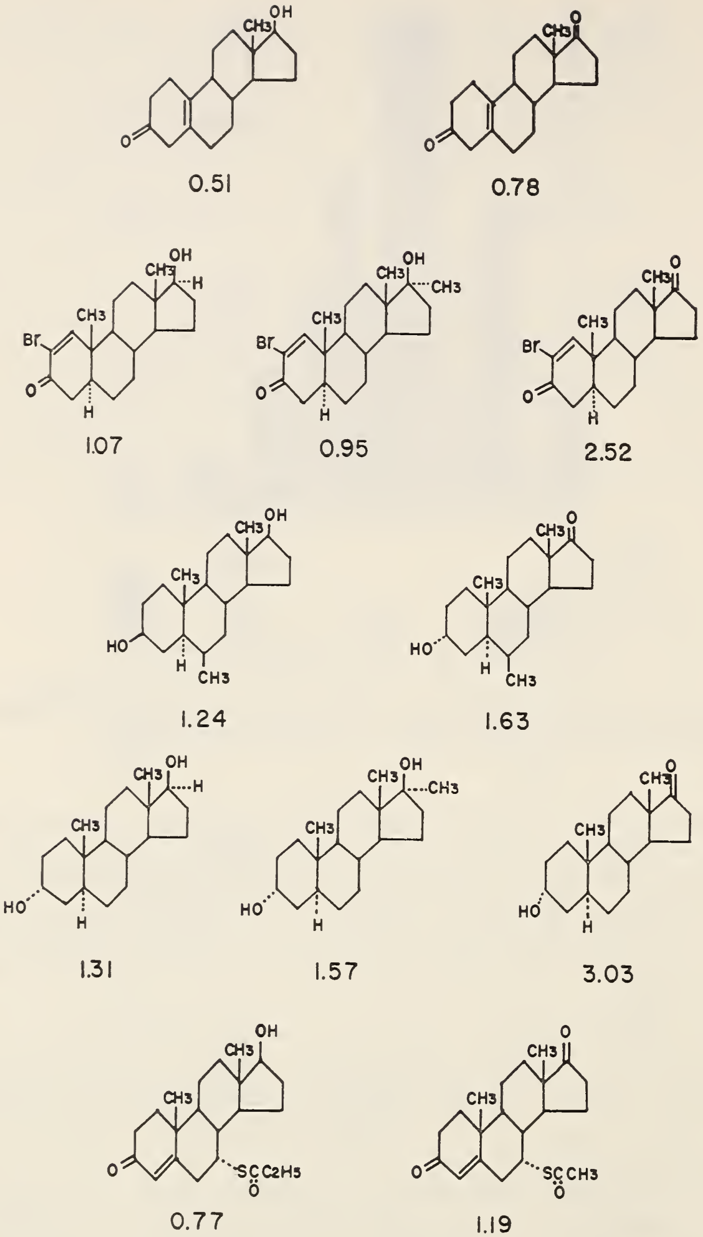
Progesterone, without either a 17-hydroxyl or a 17-ketone group, produces ventral prostate stimulation rather than seminal vesicle growth. The significance of the presence or absence of the 17-hydroxyl remains obscure.

The number of unsaturated linkages in the nucleus was next studied. From text-figure 13 it would appear that saturated compounds have greater potentiality for increasing prostate weights rather than seminal vesicles weights.

There were a number of compounds in which both the saturated and the unsaturated analogues were available. Data on some of these are presented in table 1. While usually the unsaturated compounds had lower VP/SV ratios than the corresponding saturated compounds, this shift did not always occur.

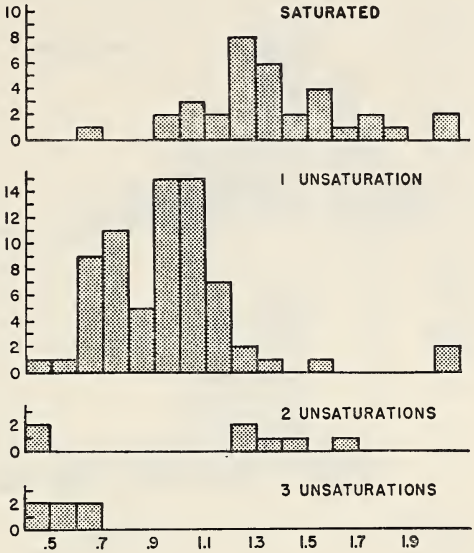
There were 94 compounds with a 17β-hydroxyl group. The significance of the 17α-substituent on these was studied. As shown in text-figure 14, there was no effect on the distribution of the VP/SV ratios when either a methyl or an ethyl group was substituted for the 17α-hydrogen.

There were 10 compounds with unsaturated side chains at the 17α-position. Three of these, with a 17α-vinyl side chain had VP/SV ratios of 0.67, 0.94, and 1.20. Four with a 17α-ethynyl side chain yielded values of 0.39, 0.65, 0.71, and 0.93, while 3 with a chlorethynyl group had values of 0.39, 0.60, and 0.63.


0.51
0.78
1.07
0.95
2.52
1.24
1.63
1.31
1.57
3.03
0.77
1.19

TEXT-FIGURE 12.—Structural formulas and VP/SV ratios of several compounds with a hydroxyl group at C-17 and their 17-keto analogues.

Text-figure 15 shows the responses to a series of testosterones with 17α-substituents. Although the ethynyl compound showed a lower potency than the ethyl, its VP/SV ratio was not appreciably lower.

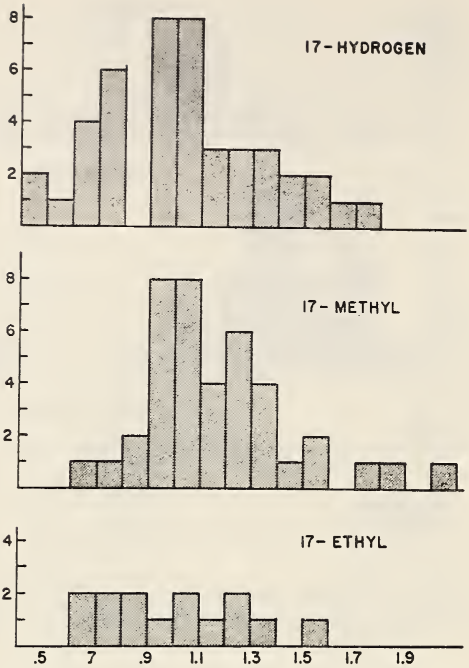


Text-figure 13.—Effect of unsaturated bonds in the steriod nucleus in the distribution of VP/SV ratios (ordinate: number of compounds).

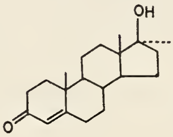
Table 1.—Effect of unsaturated bonds in the A ring on the ventral prostate and seminal vesicle responses*

Compound	Ventral prostate (mg)	Seminal vesicles (mg)	VP/SV
Androstane-3,17-diol	67. 7	51. 6	1. 31
Δ^1	62. 9	49. 7	1. 26
Androstan-17-ol-3-one	128. 5	115. 6	1. 11
Δ^1	67. 0	59. 1	1. 13
Δ^1	45. 4	50. 7	0. 90
$\Delta^{1,4}$	15. 1	10. 3	1. 47
Androstane-3-17-dione	54. 7	14. 3	3. 83
Δ^1	42. 3	22. 5	1. 88
Δ^4	35. 3	27. 0	1. 31
$\Delta^{1,4}$	13. 9	9. 4	
17α-Methylandrostan-17-ol-3-one	95. 9	72. 6	1. 32
Δ^1	68. 9	68. 4	1. 01
Δ^4	55. 0	61. 5	0. 89
$\Delta^{1,4}$	17. 4	14. 9	1. 17
17α-Ethylandrostan-17-ol-3-one	36. 2	24. 1	1. 50
Δ^1	25. 0	23. 1	1. 08
Δ^4	55. 8	53. 6	1. 04
17α-Ethyl-19-norandrostan-17-ol-3-one	26. 5	22. 2	1. 19
Δ^4	34. 8	48. 6	0. 70

*Eight rats were included in each group.



Text-figure 14.—Distribution of the VP/SV ratios for compounds having a hydrogen, methyl, or ethyl group at 17α.

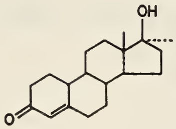


17α	VENTRAL PROSTATE	SEMINAL VESICLE	VP/SV
HYDROGEN	45.4	50.7	0.90
METHYL	55.0	61.5	0.89
ETHYL	55.8	53.6	1.04
ETHYNYL	21.5	23.0	0.93

Text-figure 15.—Ventral prostate and seminal vesicle responses to 17α-substituted testosterone.

Text-figure 16 includes a larger series of 19-nortestosterones, with both saturated and unsaturated 17α-side chains. Little androgenic response was obtained when the side chain contained more than 2 carbon atoms. The presence of unsaturated linkages in the 2-carbon side chain reduced activity but did not consistently alter the ratio.

It would appear, therefore, that the low ratios shown in text-figure 14 may be due to chance selection of this small series.



17 α	VENTRAL PROSTATE	SEMINAL VESICLE	VP/SV
HYDROGEN	20.7	29.2	0.71
METHYL	24.2	26.1	0.96
ETHYL	34.0	48.6	0.70
VINYL	17.9	19.0	0.94
ETHYNYL	12.2	17.9	0.71
PROPYL	11.8	9.5	
ALLYL	12.3	9.9	
BUTYL	10.3	9.1	
1-METHALLYL	12.0	10.8	
2-METHALLYL	8.9	8.5	

Text-figure 16.—Ventral prostate and seminal vesicle responses to 17α-substituted 19-nortestosterone (prostate and seminal vesicle weights in milligrams).

To summarize the characteristics in a molecule that appear to favor relatively greater prostate stimulation in comparison to seminal vesicle growth: 1) presence of a methyl group on C-10, 2) presence of a 17-ketone, 3) absence of unsaturated linkages in ring A.

Of less importance, but nevertheless desirable, is the presence of a ketone on C-3.

Androstanedione fulfills these requirements. A recent assay on this compound gave: ventral prostate = 54.7 mg, seminal vesicles = 14.3 mg, or a VP/SV ratio of 3.82.

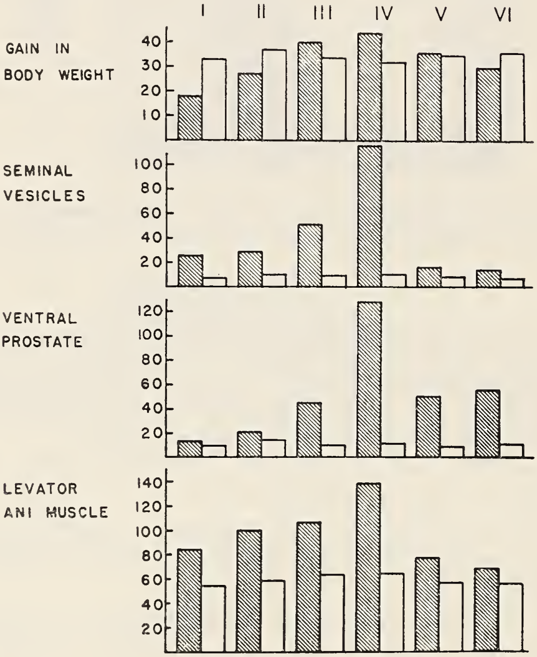
Apparently the most important characteristics in favoring seminal vesicle responses are an aromatic A ring and a 17β-hydroxyl group. Ethynyl estradiol has these characteristics. In a recent check on this compound, we obtained a ventral prostate weight of 11.9 mg and a seminal vesicle weight of 22.8 mg, giving a ratio of 0.52. Several compounds with a single unsaturation in the 5(10) position have produced ratios of this order (text-figs. 7, 9, and 12). Since estrogens, both steroidal and nonsteroidal, produce a seminal vesicle response without eliciting a ventral prostate response, one can expect a low VP/SV ratio from any estrogenic compound.

STUDIES IN INTACT RATS

We have selected a series of 6 androgens for a more detailed study. They were chosen not on the basis of their physiological importance but rather on the basis of chemical structure to illustrate the points previously mentioned. These compounds with their respective VP/SV ratios are:

I.	$\Delta^{5(10)}$-estren-17-ol-3-one	0.52
II.	19-Nortestosterone	0.71
III.	Testosterone	0.90
IV.	Dihydrotestosterone	1.10
V.	Androsterone	3.03
VI.	Androstanedione	3.82

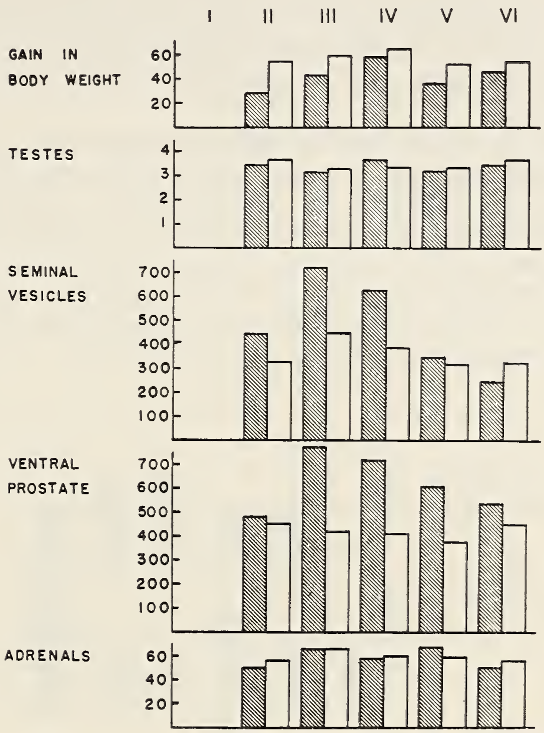
The effects of these compounds in castrated rats are shown in text-figure 17.



Text-figure 17.—Effects of a series of steroids (*see* text) on several parameters in castrated male rats. A total dose of 5 mg was administered (IM) to each of 8 rats over a period of 7 days. *Diagonal bars* represent the treated groups; *open bars*, the controls killed on the same day (body weight in grams, all others in milligrams).

Compounds I, II, III, and IV caused marked seminal vesicle responses, while compounds III, IV, V, and VI produced good stimulation of the prostate. Therefore compounds III and IV have VP/SV ratios near unity, while I and II have low, and V and VI have high ratios. The best responses in the levator ani muscle were obtained with the first 4 compounds despite the fact that compounds I and II reduced the gain in body weight.

Sex hormones can produce an inhibition of pituitary gonadotropin secretion with resultant gonadal atrophy. We studied some of these compounds in intact young adult male rats, treating daily for 14 days with a dose of 5 mg per rat per day. The results are shown in text-figure 18.



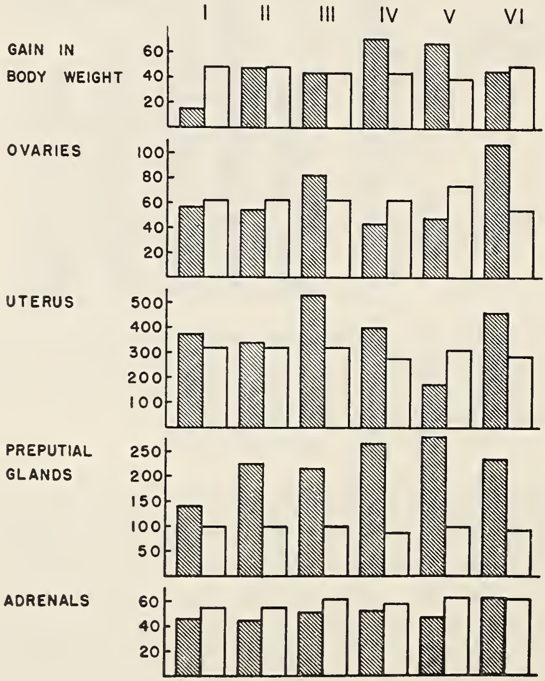
TEXT-FIGURE 18.—Effects of a series of compounds (*see* text) on several parameters in intact young adult male rats. Each rat received 5 mg of steroid daily for 14 days. *Diagonal bars* represent the treated groups; *open bars*, controls killed on the same day (body weight in grams, others in milligrams).

The depression in testis weight seen in most groups was not statistically significant. With compound VI there is a suggestion of functional inhibition in the reduced seminal vesicles. As in the castrate, compounds II, III, and IV effected marked enhancement of seminal vesicle weights and compounds III, IV, V, and VI enhanced prostatic weights. Thus in the intact rats, the ratios of VP/SV followed the pattern seen in the castrates. Body weights appear to be slightly depressed in these intact animals and there is no consistent effect on adrenal weights.

Dorfman and Shipley (*10*) compared the activity of several of these compounds on the seminal vesicles of the rat and the comb of the capon. Compound IV was twice as effective as compound III on the seminal vesicles but slightly less potent than the latter on the capon's comb Compound V had potencies relative to III of 10 percent in both tests and compound VI showed potencies of 14 and 12 percent in the 2 tests. Thus, the capon-comb test appears to correlate quite well with the rat seminal vesicle assay rather than with ventral prostate weights.

Ofner *et al.* (*11*), using direct application to the chick comb, assigned the following relative potencies to these same compounds: II, 36; III, 42; IV, 45; V, 100. The high potency of androsterone (V) in the chick-comb test does not agree with its activity on the rat seminal vesicle or ventral prostate.

In the sexually mature, intact female, we should expect some gonadal inhibition if these compounds inhibit gonadotropin secretion, and in the absence of endogenous estrogen, we should observe a decrease in uterine weight. It is well established, however, that androgens increase uterine weight even in the spayed female. In text-figure 19 are presented data we obtained after treating intact, young adult female rats. All of the compounds had an androgenic effect on the preputial gland, and except for compound I, all showed quite comparable effectiveness.



TEXT-FIGURE 19.—Effect of a series of steroids (*see* text) on several parameters in intact, young adult female rats. Each rat received 5 mg of steroid daily for 14 days. *Diagonal bars* represent the treated groups; *open bars,* controls killed on the same day (body weight in grams, others in milligrams).

Other effects of this series were quite irregular and do not appear to correlate with total androgenic activity, or specific seminal vesicle or ventral prostate-stimulating effect.

SUMMARY

We have attempted to define some characteristics of the steroid molecule that are associated with ventral prostate rather than seminal vesicle-stimulating effects. These include the presence of a keto group on C-17, a methyl group on C-10, and the absence of unsaturated linkages in ring A. We were concerned with only the ventral lobe of the rat prostate. The dorsolateral lobes and the coagulating glands were not included. Absolute potency of the various compounds was mentioned only when such potency was relative to the value of the VP/SV ratio. It was shown, however, that this ratio is quite constant even when the dosage is varied over a considerable range and thus, except at minimal response levels, the ratio is not dependent on potency. The ratio of ventral prostate to seminal vesicle response should be useful in separating classes of androgens for further study. A steroid that preferentially stimulated the prostate and had a high potency in this regard might serve as a useful tool in the evaluation of antiandrogenic compounds. Further, a steroid particularly effective in inducing seminal vesicle growth but not estrogenic offers the potential of overcoming the feminizing effects of estrogen, when the two compounds are administered concurrently, without the risk of prostatic hypertrophy.

Studies on the separation of effects at one time ascribed to a single hormone molecule should serve as a guide in the synthesis of compounds with specific activities and thus lead to the treatment and control of specific disorders without at the same time upsetting other hormonal balances.

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DISCUSSION

Dr. Williams-Ashman: I would like to inquire into the meaning of the differences in the wet weight of the rat prostate and seminal vesicles which Dr. Saunders has reported. Is it possible that the differences in the weight response of these organs to various steroids may, at least in part, reflect changes in the amount of retained secretion, rather than alterations in total cellular mass? Thus, the rat seminal vesicle has a large central lumen crammed full of secretion in adult animals after stimulation with testicular hormones, and this vesicular secretion is easily removable by manual expression. However, it is very difficult to remove all of the extracellular secretion in the lumina of the prostate gland. I wonder if, in the experiments reported, attempts were made to remove prostatic or vesicular secretions prior to measurement of the fresh weight of these organs?

Dr. Saunders: In all cases the seminal vesicles were cut in several places and the fluid expressed. However, no fluid was removed from the prostate. All the weights I have discussed were wet weights; the tissues were kept moist until weighed.

Dr. Segaloff: As I understand it, all the ratios were obtained from organ weights of rats given a standard dose of 5 mg of steroid per animal. It has been our experience that even when the seminal vesicle responds, it takes at least 10 times as much steroid for the initial response as for such response of the ventral prostate, and then the response curves are frequently not parallel. Thus the inherent androgenicity of the steroid as well as the differential effect on the organs in question would govern the size of the ratio, and we might well have an entirely different picture if another level were chosen.

Dr. Saunders: The possibility of a change in the value of the ratio at different doses was considered. With 4 compounds I presented evidence that the ratio remains more or less constant. A text-figure is presented on which are plotted a series of doses of testosterone propionate and with organ weights from the control values up to over 100 mg with the ratio remaining near unity over the entire range. Another text-figure shows testosterone, 19-nortestosterone, and $\Delta^{5(10)}$-estrenolone at several dose levels, yet the ratio for each compound remains relatively constant. With isolated cases more variability may be observed but I believe this ratio is a real measure of effect and the use of a fixed dose is justified. As to the 17-keto compounds being less potent, I think there is no doubt about this and, therefore, with a fixed dose, we are working at a lower level of response. However, as I mentioned but perhaps did not stress, we included only those compounds that had produced either seminal vesicle or ventral prostate weights of at least 15 mg. In our test method this represents an increase of 50 to 75 percent over the control value. Therefore we have eliminated from our discussion, compounds producing only minimal responses.

Dr. Mann: I wonder if an increase in weight of an accessory organ produced by administration of a chemical compound can be regarded as a general criterion of androgenic stimulation. If one injects a rabbit with diethylstilbestrol, there is a considerable hypertrophy of the prostate but the secretory function of that prostate is actually suppressed, and on closer examination one finds that it is mostly the muscle that has undergone hypertrophy, and not the secretory tissue. Is it not possible that some of the weight changes you have observed are due to an increased growth rate of elements other than the secretory tissue?

Dr. Saunders: I can lay no claim to being a histologist but I can confirm the fact, well documented in the literature, that estrogen stimulation of the seminal vesicle of the rat is observed in the connective tissue of the tubules rather than in the secretory portion of the gland. Insofar as the ventral prostate is concerned, the glands from castrated animals treated with testosterone propionate closely resemble those from intact sexually mature animals. We have not examined the glands from castrated animals treated with other steroids.

ANTIANDROGENS[1, 2]

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ANTIANDROGENS, for the purpose of this paper, are defined as substances that decrease the effectiveness of exogenously administered androgens when one uses the endpoints of seminal vesicles, prostate and/or levator ani in the castrated rat or mouse, or the comb of the chick during the first 7 to 10 days of life. The castrated rat or mouse is used so that pituitary and gonadal effects are eliminated. In the definition, the term "decrease the effectiveness" has been used and this should be defined further. The effectiveness of the androgen may be decreased by interfering with the action of androgens at the target site, but it may also be influenced by modifying the metabolism of the androgen in question. The information in this paper unfortunately does not add any evidence for or against these possibilities.

A variety of antiandrogenic compounds of diverse structures has been described. Among these compounds are estrogens (*1–4*) and carcinogenic hydrocarbons such as benzpyrene and methylcholanthrene (*5, 6*). Perhaps the most consistent group of antiandrogenic substances are those possessing progestational activity or related structurally to progesterone. In this group is the highly effective progestational agent 17-ethynyl-19-nortestosterone, which is also antiandrogenic (*5, 7, 8*). The nonhormonally active compound, 11α-hydroxyprogesterone, was reported by Byrnes *et al.* (*9*) to be an antiandrogen. A-Norprogesterone, similarly inert, was reported to be antiandrogenic by Lerner *et al.* (*10*) and this was confirmed by Dorfman and Kincl (*11*). Two progestational substances have been studied in some detail and have given a positive antiandrogenic effect in both chicks and rats. These include 19-norprogesterone and chlormadinone (6-chloro-Δ^6-dehydro-17-acetoxyprogesterone) (*11, 12*). Finally, an important antiandrogen, Ro 2-7239 (2-acetyl-7-oxo-1,2,3,4,4a,4b,5,6,-7,9,10,10a-dodecahydrophenanthrene), which in a certain sense is a contracted progesterone molecule, shows definitive androgenic effects at certain concentrations. The antiandrogenic effect of this compound

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] Supported in part by grant C-2193 from the National Cancer Institute, National Institutes of Health, Public Health Service.

has been described by various workers, including Randall and Selitto (*13*), Dorfman (*7*), and Dorfman and Stevens (*8*).

Huggins and Clark (*14*) have studied the estrogen inhibition of the androgen-maintained prostatic secretion in the castrated dog. These early studies were described by Huggins and Russell (*15*). The studies of Huggins and Clark have now been extended by Goodwin *et al.* (*4*) who showed the estrogen inhibition in androgen-maintained prostatic secretion in the hypophysectomized dog. The exact mechanism of this estrogen-androgen antagonism is not established, but it is known that the action can take place in the absence of the pituitary or the testes.

The mechanism of estrogen inhibition of androgen action may be considered from two viewpoints. There may be a direct antagonistic effect of the estrogens at the target organ, or the estrogen may act by inhibiting prolactin release by the pituitary, thus preventing the full action of testosterone. Since Goodwin *et al.* (*4*) showed that estrogen inhibition of prostatic growth can occur even in the absence of the pituitary, apparently prolactin is not an important factor.

In addition to antiandrogen studies on the dog prostate, techniques have been developed and described in detail with the use of the comb of the chick and accessory male organs in the mammal (*16*).

In the present review the results obtained by the various methods will be described and discussed.

TYPICAL RESULTS IN CHICKS

The carcinogen, methylcholanthrene, was inuncted to the comb of chicks which received either 0.06 mg of androsterone or 0.08 mg of testosterone propionate by injection. In either case highly significant decreases in comb ratios were observed, although the body weights did not change. In experiment C, which was of 12 days' duration, a total dose of 1.2 mg of either methylcholanthrene or benzpyrene inhibited the action of 0.09 mg of testosterone propionate administered by injection (*5*) (table 1).

Progesterone, by inunction, can inhibit the activity of testosterone administered in the food (table 2). The most intense response was found when 80 mg of testosterone per kg of food was fed and the comb size was increased about 5 times. Significant reduction was observed at a dose of 0.5 mg of progesterone [table 2 (*5*)].

When testosterone was fed and the test compound injected, progesterone and 17-ethynyl-19-nortestosterone were active inhibitors at 2 mg, 17-ethynyltestosterone was inactive up to 2 mg, while 9α-fluorocortisol, deoxycorticosterone, and 11β-hydroxyprogesterone were active at 0.25, 2.0, and 2.0 mg, respectively [table 3 (*5*)].

Table 4 deals with the conditions where stimulation was attained with testosterone feeding and inunction of the compounds. Significant antiandrogenic effects were noted with the following compounds at the designated minimum total doses: 17-ethynyl-19-nortestosterone (0.1 mg),

Table 1.—Methylcholanthrene and benzpyrene inhibit the action of androgens on the chick comb*

Conditions of assay:
1. Androgen injected
2. Test compounds inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Experiment (duration in days)	Androgen injected (total dose, mg)	Antiandrogen inuncted (total dose, mg)	Number of chicks	Mean comb ratio ± SE
A (7)	0	0	19	0. 43 ± 0. 02
	Androsterone (0.06)	0	16	0. 92 ± 0. 04
	Androsterone (0.06)	Methylcholanthrene (0.7)	20	0. 56 ± 0. 03
B (8)	Testosterone propionate (0.08)	0	17	0. 60 ± 0. 03
	Testosterone propionate (0.08)	Methylcholanthrene (1.2)	15	0. 44 ± 0. 03
C (12)	Testosterone propionate (0.09)		17	0. 65 ± 0. 04
	Testosterone propionate (0.09)	Methylcholanthrene (1.2)	17	0. 43 ± 0. 02
	Testosterone propionate (0.09)	Benzpyrene (1.2)	17	0. 46 ± 0. 03

*Courtesy *Acta Endocrinologica* [Kobenhavn (*5*)].

Table 2.—Progesterone inhibits the action of androgens on the comb*

Conditions of assay: 1. Testosterone fed
2. Progesterone inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Testosterone in food, mg/kg	Total dose of progesterone inuncted, mg	Number of chicks	Mean comb ratio ± SE
10	0	20	0. 61 ± 0. 03
	0. 5	19	0. 64 ± 0. 05
	1. 0	19	0. 59 ± 0. 04
	2. 0	20	0. 56 ± 0. 04
	4. 0	20	0. 47 ± 0. 03
40	0	24	1. 08 ± 0. 06
	0. 5	19	1. 29 ± 0. 06
	1. 0	20	1. 02 ± 0. 07
	2. 0	19	1. 02 ± 0. 04
	4. 0	20	0. 93 ± 0. 05
80	0	20	1. 87 ± 0. 12
	0. 5	20	1. 42 ± 0. 07
	1. 0	19	1. 38 ± 0. 07
	2. 0	19	1. 28 ± 0. 09
	4. 0	19	1. 17 ± 0. 06

* Courtesy *Acta Endocrinologica* [Kobenhavn (*5*)].

Table 3.—Various steroids that inhibit the action of androgens on the chick comb*

Conditions of assay: 1. Testosterone fed to all chicks at level of 80 mg/kg
2. Test compounds injected
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Steroid injected	Total dose, mg	Number of chicks	Mean comb ratio ± SE
0	0	40	1.90 ± 0.09
Progesterone	0.5	15	1.85 ± 0.09
	1.0	36	1.76 ± 0.10
	2.0	37	1.74 ± 0.08
17-Ethynyl-19-nortestosterone	0.25	22	1.67 ± 0.12
	0.50	37	1.64 ± 0.08
	1.0	14	1.79 ± 0.17
	2.0	37	1.71 ± 0.09
17-Ethynyltestosterone	0.5	15	2.16 ± 0.14
	1.0	15	1.84 ± 0.14
	2.0	15	1.91 ± 0.19
9α-Fluorocortisol	0.25	19	1.57 ± 0.12
	0.50	20	1.50 ± 0.10
	2.00	21	1.48 ± 0.11
Deoxycorticosterone	0.5	15	1.73 ± 0.13
	1.0	15	1.76 ± 0.14
	2.0	15	1.52 ± 0.15
11β-Hydroxyprogesterone	2.0	28	0.92 ± 0.05
	4.0	24	0.74 ± 0.03

*Courtesy *Acta Endocrinologica* [Kobenhavn (*5*)].

17-ethynyltestosterone (1 mg), cortisol (0.25 mg), 9α-fluorocortisol (0.1 mg), diethylstilbestrol (0.1 mg), and estradiol-17β (0.5 mg).

TABLE 4.—Various steroids that inhibit the action of androgens on the chick comb*

Conditions of assay:
1. Testosterone fed at levels of 80 mg/kg
2. Test compound inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Steroid inuncted	Total dose, mg	Number of chicks	Mean comb ratio ± SE
0	0	396	1.75 ± 0.02
Progesterone	0.5	61	1.54 ± 0.05
	1.0	211	1.41 ± 0.03
	2.0	216	1.38 ± 0.03
	4.0	63	1.15 ± 0.04
17-Ethynyl-19-nortestosterone	0.1	138	1.40 ± 0.03
	0.2	97	1.35 ± 0.03
	0.4	77	1.19 ± 0.04
	0.8	56	1.25 ± 0.06
	1.0	33	1.34 ± 0.09
	1.6	43	1.21 ± 0.04
17-Ethynyltestosterone	1.0	36	1.50 ± 0.08
	2.0	37	1.66 ± 0.08
	4.0	20	1.15 ± 0.09
Cortisol	0.25	22	1.67 ± 0.12
	0.50	53	1.56 ± 0.07
	1.00	29	1.21 ± 0.07
	2.00	22	1.56 ± 0.09
9α-Fluorocortisol	0.10	13	1.36 ± 0.13
	0.20	15	1.51 ± 0.14
	1.00	15	1.32 ± 0.07
	2.00	13	1.26 ± 0.07
Diethylstilbestrol	0.1	27	1.51 ± 0.07
	0.5	62	1.66 ± 0.09
	1.0	60	1.63 ± 0.06
	2.0	32	1.62 ± 0.08
Estradiol-17β	0.5	17	1.13 ± 0.08
	1.0	15	0.95 ± 0.08
	2.0	18	1.33 ± 0.10

*Courtesy *Acta Endocrinologica* [Kobenhavn (*5*)].

Steviol at the total dose of 3 mg was antiandrogenic in the chick comb test, and dihydroisosteviol was active at 1.5 mg. In these tests (table 5) the stimulation was effected by a single injection of long-acting testosterone enanthate and the inunction of the test compounds (*17*). Under the same conditions, A-norprogesterone (table 6) and 19-norprogesterone (table 7) were found to be active antiandrogens by inunction to the comb (*11*).

A detailed study of the inhibitory activity by subcutaneous injection is summarized in table 8. The chicks showed no changes in body weight.

TABLE 5.—Dihydroisosteviol inhibits the action of testosterone on the chick comb*

Conditions of assay:
1. Testosterone enanthate injected
2. Dihydroisosteviol inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Experiment No.	Compound inuncted (total dose, mg)	Total dose of testosterone enanthate, mg	Number of chicks	Mean comb ratio ± SE
A	0	0	25	0. 37 ± 0. 018
	0	0. 5	25	0. 92 ± 0. 039
	Norethisterone (0.25)	0. 5	25	0. 64 ± 0. 028
	Norethisterone (1.0)	0. 5	25	0. 50 ± 0. 019
	Steviol (0.5)	0. 5	10	0. 88 ± 0. 071
	Steviol (2.0)	0. 5	10	0. 84 ± 0. 050
B	0	0	20	0. 33 ± 0. 012
	0	0. 5	20	0. 86 ± 0. 039
	Norethisterone (0.25)	0. 5	20	0. 72 ± 0. 028
	Norethisterone (1.0)	0. 5	19	0. 59 ± 0. 024
	Steviol (2.0)	0. 5	10	0. 74 ± 0. 940
C	0	0	20	0. 47 ± 0. 015
	0	0. 5	20	1. 09 ± 0. 074
	Norethisterone (0.25)	0. 5	20	0. 97 ± 0. 034
	Norethisterone (1.0)	0. 5	20	0. 80 ± 0. 048
	Dihydroisosteviol (0.5)	0. 5	10	0. 98 ± 0. 029
	Dihydroisosteviol (3.0)	0. 5	10	0. 77 ± 0. 072
	Steviol (0.5)	0. 5	10	1. 03 ± 0. 074
	Steviol (3.0)	0. 5	9	0. 92 ± 0. 055
D	0	0	15	0. 35 ± 0. 022
	0	0. 5	14	0. 90 ± 0. 051
	Ro 2-7239 (0.5)	0. 5	14	0. 73 ± 0. 045
	Ro 2-7239 (1.5)	0. 5	14	0. 46 ± 0. 016
	Dihydroisosteviol (1.5)	0. 5	14	0. 77 ± 0. 049
	Dihydroisosteviol (3.0)	0. 5	14	0. 70 ± 0. 041

*Courtesy *Endocrinology* (17).

Norethisterone and Ro 2-7239 were highly active, with norethisterone being significantly the more effective. Both compounds were reasonably active at a total dose of 0.1 mg. Cortisol was effective at 0.5 mg, MER-

TABLE 6.—A-Norprogesterone inhibits the action of testosterone*

Conditions of assay:
1. Testosterone enanthate injected
2. A-Norprogesterone inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Test material	Total dose, mg	Testosterone enanthate (total dose, mg)	Number of chicks	Mean comb ratio ± SE
0	0	0	22	0.54 ± 0.026
0	0	0.5	19	1.32 ± 0.082
Ro 2-7239	0.25	0.5	19	0.97 ± 0.058
	0.5	0.5	20	0.88 ± 0.040
	1.0	0.5	20	0.87 ± 0.030
	2.0	0.5	20	0.84 ± 0.051
	4.0	0.5	19	1.12 ± 0.058
A-Norprogesterone	0.25	0.5	19	1.19 ± 0.054
	0.5	0.5	18	1.27 ± 0.066
	1.0	0.5	20	1.22 ± 0.055
	2.0	0.5	20	0.98 ± 0.036
	4.0	0.5	18	0.99 ± 0.038

*Dorfman and Kincl (*11*).

TABLE 7.—19-Norprogesterone inhibits the activity of testosterone on the chick comb*

Conditions of assay:
1. Testosterone enanthate injected
2. 19-Norprogesterone inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Experiment No.	Designation	Total dose, mg	Testosterone enanthate (total dose, mg)	Number of chicks	Mean comb ratio ± SE
A	0	0	0	8	0.25 ± 0.015
	0	0	0.5	8	0.64 ± 0.098
	Ro 2-7239	1	0.5	10	0.37 ± 0.029
	19-Norprogesterone	3	0.5	10	0.40 ± 0.032
B	0	0	0	10	0.36 ± 0.028
	0	0	0.5	12	0.79 ± 0.061
	Ro 2-7239	0.25	0.5	8	0.66 ± 0.082
		1.0	0.5	10	0.57 ± 0.053
	19-Norprogesterone	1.0	0.5	10	0.82 ± 0.079
		2.0	0.5	10	0.74 ± 0.058
		3.0	0.5	9	0.90 ± 0.087
C	0	0	0.5	7	0.40 ± 0.023
	0	0	0.5	10	0.81 ± 0.042
	Ro 2-7239	0.25	0.5	10	0.79 ± 0.046
		0.5	0.5	10	0.68 ± 0.055
		1.0	0.5	10	0.65 ± 0.041
	19-Norprogesterone	1.0	0.5	10	0.88 ± 0.041
		3.0	0.5	10	0.61 ± 0.049

*Dorfman and Kincl (*11*).

25 [3] showed a borderline response at 16 mg, and progesterone was effective at a total dose of 4.5 mg (*18*).

TABLE 8.—Various compounds inhibit the activity of testosterone on the chick comb*

Conditions of assay: 1. Testosterone enanthate by injection
2. Test compound by injection
3. 7-Day test

Compound	Total dose, mg	Number of chicks	Comb inhibition (%)
Norethisterone	0. 1	57	38
	0. 25	56	40
	0. 5	129	58
	1. 0	55	63
	1. 5	73	45
	2. 0	55	66
	4. 0	55	67
	4. 5	71	50
Ro 2-7239	0. 1	56	24
	0. 25	56	16
	0. 5	127	27
	1. 0	56	38
	1. 5	72	34
	2. 0	28	61
	4. 0	56	54
	4. 5	74	43
Cortisol	0. 5	73	20
	1. 5	76	22
	4. 5	73	30
MER-25	0. 5	74	6
	4. 5	73	16
Progesterone	0. 5	76	29
	1. 5	74	35
	4. 5	74	40

*Dorfman (*18*).

Table 9 clearly demonstrates that the androgenic activity of orally administered androst-4-ene-3,17-dione may be inhibited by Ro 2-7239. The activity of testosterone administered in the same way is also blocked. This report (*18*) shows that the same result can be obtained if methyltestosterone is used instead of testosterone in the oral feeding.

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[3] MER-25 = phenethyl alcohol, α-[*p*-(2-diethylaminoethoxy)-phenyl]-*p*-methoxy-α-phenyl.

TABLE 9.—Ro 2-7239 inhibits the activity of androst-4-ene-3,17-dione on the chick comb*

Conditions of assay:
1. Androst-4-ene-3,17-dione fed to all chicks in food (80 mg/kg)
2. Ro 2–7239 inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Material administered	Total dose, mg	Number of chicks	Mean comb ratio ± SE
0	0	24	1. 31 ± 0. 054
Ro 2-7239	0. 25	19	0. 98 ± 0. 053
	0. 5	20	1. 02 ± 0. 053
	1. 0	20	0. 95 ± 0. 062
	2. 0	20	0. 77 ± 0. 047

*Courtesy *Endocrinology* (*8*).

TABLE 10.—Ro 2-7239 inhibits the activity of methyltestosterone on the chick comb*

Conditions of assay:
1. Methyltestosterone fed in food to all chicks (10 mg/kg)
2. Ro 2-7239 inuncted
3. 7-Day test
4. Comb ratio = mg of comb per g of body weight

Material administered	Total dose, mg	Number of chicks	Mean comb ratio ± SE
0	0	14	0. 55 ± 0. 027
Ro 2-7239	0. 5	14	0. 53 ± 0. 040
	1. 0	14	0. 49 ± 0. 028
	2. 0	13	0. 42 ± 0. 025

*Courtesy *Endocrinology* (*8*).

TYPICAL RESULTS IN MAMMALS

Antiandrogenic activity has been found in a variety of steroids and a few nonsteroids through the use of assays in rats and mice in addition to the examples on the chick comb presented in tables 1 through 10. Data concerning the mammalian indicators with the use of the androgen-stimulated castrated rat or the castrated mouse are presented in tables 11 through 17.

Ro 2-7239 administered by injection produced inhibition of either testosterone or androst-4-ene-3,17-dione stimulation (table 11), and the compound was also active when administered by gavage (table 12). Table 13 deals with 19-norprogesterone, which also was an effective inhibitor by injection (*11*).

TABLE 11.—Ro 2-7239 administered by injection inhibits the action of androgens in the castrated rat*

Conditions of assay: 1. Androgen injected
2. Ro 2-7239 injected
3. 7-Day test

T = Testosterone
A = Androst-4-ene-3,17-dione
R = Ro 2-7239

Experiment No.	Material administered	Total dose, mg	Number of rats	Mean tissue ratio ± SE		
				Seminal vesicle	Prostate	Levator ani
A	0	0	7	0. 05 ± 0. 005	0. 07 ± 0. 007	0. 24 ± 0. 031
	A	8	6	0. 50 ± 0. 049	0. 72 ± 0. 052	0. 46 ± 0. 027
	A R	8 50	7	0. 30 ± 0. 020	0. 42 ± 0. 026	0. 48 ± 0. 053
	T	2	7	0. 94 ± 0. 058	0. 96 ± 0. 072	0. 67 ± 0. 045
	T R	2 50	7	0. 68 ± 0. 053	0. 65 ± 0. 031	0. 57 ± 0. 026
B	0	0	10	0. 07 ± 0. 004	0. 08 ± 0. 009	0. 23 ± 0. 012
	A	8	10	0. 37 ± 0. 051	1. 01 ± 0. 066	0. 46 ± 0. 066
	A R	8 50	10	0. 34 ± 0. 027	0. 98 ± 0. 054	0. 39 ± 0. 025
	A R	8 150	10	0. 25 ± 0. 011	0. 67 ± 0. 062	0. 45 ± 0. 024
	T	2	8	0. 90 ± 0. 100	1. 19 ± 0. 061	0. 63 ± 0. 34
	T R	2 50	9	0. 81 ± 0. 087	1. 00 ± 0. 041	0. 59 ± 0. 037

	T R	2 150	10	0.67 ± 0.087	0.93 ± 0.041	0.57 ± 0.034
C	0	0	8	0.11 ± 0.004	0.14 ± 0.015	0.29 ± 0.009
	A	8	9	0.80 ± 0.075	1.23 ± 0.111	0.56 ± 0.036
	A R	8 50	10	0.50 ± 0.046	1.21 ± 0.108	0.48 ± 0.025
	A R	8 150	10	0.40 ± 0.037	0.85 ± 0.082	0.56 ± 0.023
	T	2	10	1.11 ± 0.080	1.40 ± 0.088	0.74 ± 0.043
	T R	2 50	10	0.94 ± 0.056	1.09 ± 0.059	0.67 ± 0.021
	T R	2 150	10	0.83 ± 0.057	0.90 ± 0.065	0.65 ± 0.028
D	0	0	15	0.14 ± 0.007	0.16 ± 0.10	0.21 ± 0.015
	T	2.4	15	1.96 ± 0.10	1.35 ± 0.12	0.55 ± 0.005
	T R	2.4 0.5	14	1.61 ± 0.12	1.40 ± 0.14	0.57 ± 0.031
	T R	2.4 1.0	15	1.32 ± 0.099	1.15 ± 0.076	0.56 ± 0.034
	T R	2. 4.0	14	1.15 ± 0.062	1.04 ± 0.073	0.49 ± 0.027
	T R	2.4 10.0	14	1.44 ± 0.066	1.26 ± 0.056	0.58 ± 0.032

*Courtesy *Endocrinology* (8).

TABLE 12.—Ro 2–7239 administered by gavage inhibits the activity of testosterone in the castrated rat

Conditions of assay:
1. Testosterone injected
2. Ro 2–7239 administered by gavage
3. 7-Day test

Total dose of Ro 2–7239 by gavage, mg	Total dose of testosterone by injection, mg	Number of rats	Mean tissue ratio ± SE		
			Seminal vesicle	Prostate	Levator ani
0	0	8	0. 10 ± 0. 007	0. 16 ± 0. 009	0. 21 ± 0. 013
0	2. 4	9	1. 03 ± 0. 075	1. 24 ± 0. 082	0. 40 ± 0. 017
100	2. 4	10	0. 97 ± 0. 087	0. 98 ± 0. 062	0. 43 ± 0. 017

Dorfman (*12*, *19*) studied the following compounds, mostly steroids, for possible antiandrogenic activity in the testosterone-stimulated, immature castrated rat using the indicators of prostate, seminal vesicles, and levator ani:

I. $\Delta^{4,17(20)}$-Cis pregnadien-21-oic acid,3,11-diketo methyl ester
II. 17α-Hydroxyprogesterone hexanoate
III. 17α-Hydroxy-Δ^4 -pregnene-3,11,20-trione
IV. 2α-Methyl-17β-hydroxy-19-nor-Δ^4-androsten-3-one
V. $\Delta^{4,16}$-Pregnadiene-3,11,20-trione
VI. 9α-Chloro-17β-hydroxy-17α-methyl-Δ^4-androsten-3-one
VII. 21-Fluoro-Δ^4-pregnene-3,20-dione
VIII. 21-Fluoro-11β-hydroxy-Δ^4-pregnene-3,20-dione
IX. 17α-Acetoxyprogesterone
X. 6α-Methyl-17α-acetoxyprogesterone
XI. Cholesterol
XII. 11α-Hydroxyprogesterone
XIII. 16,17-Epoxyprogesterone
XIV. 19-Norprogesterone
XV. Progesterone
XVI. Pregnenolone
XVII. 16,17-Epoxy-21-acetoxy-Δ^4-pregnene-3,20-dione
XVIII. 16,17-Epoxy-3β-acetoxy-Δ^5-pregnen-20-one
XIX. Civetone
XX. 3β-Hydroxy-Δ^4-pregnen-20-one,20-ethylene ketal
XXI. 6-Chloro-Δ^6-dehydro-17-acetoxyprogesterone
XXII. 6-Chloro-Δ^6-dehydro-17-hydroxyprogesterone
XXIII. Ro 2-7239
XXIV. A-Norprogesterone
XXV. 17α-Hydroxyprogesterone
XXVI. Clomiphene
XXVII. 6β-Hydroxytestosterone
XXVIII. Civetolone
XXIX. 6α-Chloro-17-acetoxyprogesterone
XXX. Cinophen
XXXI. Aminopyrine
XXXII. Neomycin sulfate

Table 13.—The antiandrogenic activity of 19-norprogesterone (19 NP) in the castrated rat*

Conditions of assay:
1. Androgen injected
2. Ro 2–7239 injected
3. 7-Day test

Test material designation	Total dose, mg	Testosterone (total dose, mg)	Number of rats	Mean body weight, g	Mean tissue ratio ± SE		
					Prostate	Seminal vesicle	Levator ani
0	0	0	6	97	0. 15 ± 0. 017	0. 12 ± 0. 006	0. 24 ± 0. 007
0	0	2	6	89	1. 33 ± 0. 119	1. 16 ± 0. 120	0. 54 ± 0. 052
19 NP	150	0	6	97	0. 20 ± 0. 022	0. 16 ± 0. 004	0. 34 ± 0. 020
19 NP	150	2	6	110	0. 91 ± 0. 119	0. 90 ± 0. 121	0. 39 ± 0. 020
0	0	0	6	87	0. 11 ± 0. 007	0. 10 ± 0. 004	0. 27 ± 0. 019
0	0	2	6	98	1. 08 ± 0. 110	0. 77 ± 0. 080	0. 64 ± 0. 040
19 NP	150	2	4	106	0. 83 ± 0. 139	0. 53 ± 0. 089	0. 52 ± 0. 031
0	0	0	6	89	0. 12 ± 0. 017	0. 08 ± 0. 004	0. 25 ± 0. 027
0	0	2	6	96	1. 31 ± 0. 091	0. 99 ± 0. 084	0. 73 ± 0. 058
19 NP	150	2	5	109	0. 65 ± 0. 086	0. 46 ± 0. 036	0. 44 ± 0. 031
0	0	0	7	84	0. 14 ± 0. 015	0. 09 ± 0. 004	0. 31 ± 0. 007
0	0	2	7	91	1. 02 ± 0. 101	0. 70 ± 0. 120	0. 56 ± 0. 040
19 NP	100	0	7	82	0. 16 ± 0. 017	0. 10 ± 0. 007	0. 27 ± 0. 020
19 NP	100	2	7	83	0. 82 ± 0. 039	0. 51 ± 0. 059	0. 50 ± 0. 040

*Dorfman and Kincl (*11*).

Table 14.—Relative potency of various antiandrogenic compounds*

(Relative potency listed in order of decreasing activity)

On basis of prostate		On basis of seminal vesicles		On basis of levator ani	
Compound No.	Minimum dose (% inhibition)	Compound No.	Minimum dose (% inhibition)	Compound No.	Minimum dose (% inhibition)
XXIII	20 (38)	XXIII	20 (38)	XV	50 (33)
XXI	50 (31)	IV	25 (33)	VII	50 (27)
VI	50 (30)	V	25 (31)	XXI	50 (27)
VII	50 (29)	VI	50 (30)	X	50 (22)
III	50 (27)	XXI	50 (30)	III	50 (20)
VIII	50 (20)	XIV	50 (29)	VIII	50 (20)
V	50 (19)	VIII	50 (29)	II	50 (15)
I	50 (15)	XXII	50 (28)	XXIII	50 (10)
II	50 (14)	VII	50 (26)	XIV	110 (25)
XIII	100 (26)	X	50 (21)	XXIV	150 (59)
XXIV	100 (23)	XV	50 (21)	XII	150 (44)
XII	150 (37)	III	50 (19)	XXXI	150 (38)
XIV	150 (36)	IX	50 (15)	XXII	150 (37)
		XIII	100 (43)	V	150 (33)
		XXIV	100 (31)	XXXII	150 (27)
		XII	150 (49)	I	150 (9)
		XXXI	150 (30)	XIII	200 (18)
		XXXII	150 (30)		
		II	150 (29)		
		XXX	150 (28)		
		I	150 (16)		

* Dorfman (*12*, *19*)

TABLE 15.—Compounds inactive in a rat antiandrogen assay*

On basis of prostate		On basis of seminal vesicles		On basis of levator ani	
Compound	Dose, mg	Compound	Dose, mg	Compound	Dose, mg
XVI	500	XVI	500	XVI	500
XI	250	XI	250	XI	250
XXV	200	XXV	200	XXV	200
XX	150	XX	150	IV	150
XXII	150	XXIX	150	VI	150
XXIX	150	XVII	125	IX	150
XXX	150	XVIII	125	XXIX	150
XXXI	150	XXVIII	100	XXX	150
IV	150	XXVII	40	XX	150
IX	150	XXVI	25	XVII	125
XXXII	150	XIX	25	XVIII	125
XVII	125			XXVIII	100
XVIII	125			XXVII	40
XXVIII	100			XXVI	25
XXVII	40			XIX	25
XXVI	25				
XIX	25				

*Dorfman (*12, 19*).

Twenty-one compounds were active on the basis of the seminal vesicle response, 16 inhibited the action of testosterone on the prostate, and 17 prevented the action of testosterone on the levator ani muscle (table 14). The inactive compounds are listed in table 15.

Antiandrogenic activity has been observed on all three indicators (seminal vesicle, prostate, and levator ani) for Ro 2-7239, 6-chloro-Δ^6-dehydro-17-acetoxyprogesterone, A-norprogesterone, 19-norprogesterone, 11α-hydroxyprogesterone, 9α-chloro-17α-methyltestosterone, 21-fluoroprogesterone, 11-keto-17α-hydroxyprogesterone, 11β-hydroxy-21-fluoroprogesterone, 11-keto-Δ^{16}-dehydroprogesterone, $\Delta^{4,17(20)}$-cis-pregnadien-21-oic acid-3,11-diketo methylester, 17α-hydroxyprogesterone hexanoate, 16,17-epoxyprogesterone, 6α-methyl-17-acetoxyprogesterone, and progesterone.

A total dose of 2 mg of testosterone injected in a carboxymethylcellulose suspension was inhibited by 100 mg doses of each of 3 different compounds (table 16). The solvent-injected control group had prostate and seminal vesicle ratios of 0.08 ± 0.006 and 0.08 ± 0.004, respectively. Testosterone at a total dose of 2 mg did not produce a significant difference in body weight, but did produce a fourfold increase in the prostate ratio and a 12-fold increase in the seminal vesicle ratio. One hundred mg of A-norprogesterone did not decrease the prostatic ratio singificantly. However, the ratio of the seminal vesicles decreased from 1.10 ± 0.15 to 0.64 ± 0.11. Progesterone caused a similar but somewhat more intense response at the same dose level. Ro 2-7239, at the total dose of 100 mg, inhibited the action of testosterone on the prostate and seminal vesicle ratios.

Table 17 shows a series of experiments on the influence of progesterone on the action of testosterone when both compounds were administered in sesame oil solution. In these experiments 0.4 and 0.8 mg of testosterone

Table 16.—The antiandrogenic activity of various steroids in a castrated mouse assay*

Conditions of assay:
1. Mice castrated at 21 to 23 days of age
2. Testosterone injected once daily in 0.1 ml of carboxymethylcellulose
3. Test material injected once daily in 0.2 ml of carboxymethylcellulose at a site different from that for testosterone
4. 7-Day test—autopsy 24 hours after last injection

Material injected					Tissue ratio ± SE	
Designation	Total dose, mg	Testosterone injected, mg	Number of mice	Mean body weight, g	Prostate	Seminal vesicles
0	0	0	10	16	0. 08 ± 0. 006	0. 08 ± 0. 004
0	0	2	9	18	0. 35 ± 0. 03	1. 10 ± 0. 15
A-Norprogesterone	100	2	9	16	0. 34 ± 0. 04	0. 64 ± 0. 11
Progesterone	100	2	9	16	0. 29 ± 0. 03	0. 40 ± 0. 05
Ro 2-7239	100	2	6	16	0. 24 ± 0. 02	0. 56 ± 0. 03

* Dorfman (*20*).

Table 17.—The antiandrogenic activity of various steroids in a castrated mouse assay*

Conditions of assay:
1. Mice castrated at 21 to 23 days of age
2. Testosterone injected once daily in 0.1 ml of sesame oil
3. Test material injected once daily in 0.2 ml of sesame oil
4. 7-Day test—autopsy 24 hours after last injection

Experiment No.	Material injected	Total dose, mg	Testosterone injected, mg	Number of mice	Mean body weight, g	Tissue ratio ± SE	
						Prostate	Seminal vesicles
A	0	0	0	8	18	0. 07 ± 0. 008	0. 13 ± 0. 014
	0	0	0. 4	10	19	0. 21 ± 0. 019	0. 62 ± 0. 057
	Progesterone	10	0. 4	10	18	0. 22 ± 0. 025	0. 70 ± 0. 077
		20	0. 4	10	14	0. 30 ± 0. 052	0. 73 ± 0. 069
		40	0. 4	7	17	0. 20 ± 0. 018	0. 46 ± 0. 049
B	0	0	0	10	18	0. 19 ± 0. 032	0. 24 ± 0. 025
	0	0	0. 4	7	17	0. 33 ± 0. 047	0. 93 ± 0. 122
	Progesterone	10	0. 4	10	20	0. 34 ± 0. 027	0. 77 ± 0. 064
		20	0. 4	8	17	0. 38 ± 0. 041	0. 73 ± 0. 066
		40	0. 4	10	22	0. 19 ± 0. 018	0. 37 ± 0. 026
C	0	0	0	8	18	0. 07 ± 0. 008	0. 13 ± 0. 014
	0	0	0. 8	8	17	0. 23 ± 0. 025	0. 85 ± 0. 068
	Progesterone	10	0. 8	9	18	0. 26 ± 0. 017	0. 81 ± 0. 038
		20	0. 8	9	17	0. 24 ± 0. 017	0. 72 ± 0. 035
		40	0. 8	8	18	0. 18 ± 0. 012	0. 47 ± 0. 080
D	0	0	0	10	18	0. 19 ± 0. 032	0. 24 ± 0. 025
	0	0	0. 8	9	20	0. 36 ± 0. 047	0. 90 ± 0. 088
	Progesterone	10	0. 8	5	19	0. 34 ± 0. 060	0. 65 ± 0. 092
		20	0. 8	10	22	0. 33 ± 0. 038	0. 71 ± 0. 081
		40	0. 8	10	19	0. 26 ± 0. 023	0. 46 ± 0. 047

*Dorfman (*20*).

were injected, and the dose of progesterone ranged from 10 to 40 mg. No significant antiandrogenic effect was elicited on the prostate at the 10, 20, or 40 mg dose levels of progesterone in experiment A. The 10 and 20 mg doses of progesterone were ineffective, but the total dose of 40 mg produced a highly significant inhibition of seminal vesicle ratio from 0.62 ± 0.057 to 0.46 ± 0.049. In experiment B (table 17) the 0.4 ml of testosterone injected over the 7-day period caused a significant increase in the prostate and seminal vesicle ratios. The prostate ratio was increased almost twofold, while the seminal vesicle ratio was increased something less than fourfold. In this experiment as little as 10 mg per day of progesterone significantly decreased the seminal vesicle ratio from 0.93 ± 0.122 to 0.77 ± 0.064, while the prostatic ratio remained unchanged. At the 20 mg dose in experiment B no change in prostate ratio was found, but again the seminal vesicle ratio was decreased. At 40 mg, however, a significant decrease in prostatic and seminal ratios was observed. Experiment C was essentially similar to experiment A, and experiment D was similar to experiment B.

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DISCUSSION

Dr. Segaloff: Our findings with this type of assay are in essential agreement. However, we emphasized a different type of assay in our search for androgen antagonists. I believe that we should use androgen levels that can be completely inhibited with available compounds and then raise the level of administered androgen as the compounds are improved. Such levels are but a small fraction of those given by Dr. Dorfman. In our inbred Fischer rats, 1 μg of testosterone daily doubles the ventral prostate weight in 7 days. We have now found a few compounds that produce complete inhibition in such an assay. They also produce inhibition in Dr. Dorfman's types of assay. These compounds are rather different from those discussed by him.

Since the 17β-hydroxyl group contributes so much to androgenicity, it must be removed in androgen antagonists. However, simple removal of this oxygen function does not suffice. One must replace the hydroxyl with a blocking group. For this we have used 17β-alkyl substitution. We initially thought that it would be necessary also to remove the C-18 methyl group to assist in holding the 17β-alkyl against an enzyme's reactive site. Such a compound (17,17-dimethyl-18-nor-4,13(14)-androstadien-3-one) was prepared from methyltestosterone by the Wagner-Meerwein rearrangement and proved effective. Indeed, this compound has temporarily lowered the acid phosphatase in a few patients with prostatic carcinoma.

After discussing the problem with Dr. Dorfman and others, we decided to see if it was necessary to remove the C-18 methyl group. Preliminary studies indicate that 17β-methyl or 17β-ethyl steroids show antiandrogenicity even when C-18 is not removed. Thus I believe that the 17β-alkyl steroids constitute a new class of androgen antagonists.

Dr. Mason: We are very interested in antiandrogen assays and think we can improve the sensitivity of the chick antiandrogen assay. We use a very fine needle to introduce the testosterone enanthate directly under the chick comb. During the 7 days of the assay two injections are given with a total dose of 0.1 mg of testosterone enanthate. The material tested for antiandrogenicity is also given in two injections, subcutaneously, behind the wing. We believe that we get more reliable inhibition with subcutaneous injections than with inunction. We have had good inhibition with both norethisterone and progesterone. The low dose of the stimulator (0.1 mg testosterone enanthate) allows the test compound to be used at a higher ratio without toxicity. We have no idea why progesterone acts as an antiandrogen in the chick comb assay but has no effect in the dog prostatic secretion test. If anybody can explain why there is this variance, I would be happy to hear it.

Dr. Jensen: I would like to ask Dr. Mason whether any of these antiandrogens, described by Dr. Dorfman and by Dr. Segaloff as being effective in the rat or the chick, show any ability to inhibit prostatic secretion in the dog.

Dr. Mason: One that I do remember is the A-norprogesterone which we gave in amounts up to 1 g per day, for 10 days, with practically no effect. We have tried this in 4 dogs at two different levels and got little effect. Also, I do not believe that our results with the rat have revealed any significant antiandrogen activity. I have seldom seen inhibition greater than 20 or 25 percent. Whenever we have gotten inhibition we have usually gotten considerable drop in body weight, and I do think that it would be worthwhile for the Cancer Chemotherapy National Service Center to take our data and correlate body weights with prostatic and seminal vesicle weights to come up with a statistical evaluation of this whole problem.

Dr. Grayhack: Dr. Dorfman, does progesterone in your experience stimulate the accessory sex glands of the rat? If it does, how do you explain the antiandrogenic effect that you see?

Dr. Dorfman: Yes, progesterone at high doses does possess some androgenic activity. The finding of weak androgens that are also antiandrogenic has been observed before, for example, the compound Ro 2-7239. Perhaps the antiandrogen mechanism involves the weak androgen's successful competition for the site where the strong androgen needs to attach for its biological effect.

HUMAN PITUITARY INTERSTITIAL CELL-STIMULATING HORMONE[1, 2]

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Among the pituitary hormones, interstitial cell-stimulating hormone (ICSH) is the most active agent responsible for the growth and development of the male prostate gland. The biological potency of ICSH depends upon the production of androgen(s) from the interstitial cells of the testes as stimulated by ICSH. In the absence of the testes, ICSH has no effect on the accessory reproductive organs.

ICSH is not the only hormone in the pituitary which exerts an influence on the male accessory glands. Some years ago, with A. J. Lostroh, we observed that growth hormone (GH) exhibits a slight but definitive effect (*1*) on the growth of the prostate gland in hypophysectomized-castrated male rats (table 1). Moreover, GH acts as a synergist (*2*, *3*) with ICSH in hypophysectomized rats or mice (table 2). This synergistic action of GH is not surprising, since it was observed, for the first time in 1955 by Huggins *et al.* (*4*), that GH enhances the effect of testosterone propionate on the weights of the ventral prostates in young hypophysectomized-castrated Sprague-Dawley rats.

Table 1.—Effect of bovine growth hormone on the weight of ventral prostate of hypophysectomized-castrated male rats*

Rats†	Daily dose (mg)	Number of rats	Body weight Onset (g)	Body weight Autopsy (g)	Ventral prostate (mg)
Long-Evans	0	8	109	112	12.6 ± 0.5‡
	0.25	4	116	129	14.5 ± 1.8
	0.50	8	125	148	16.3 ± 0.5 ($P<0.001$)
Sprague-Dawley	0	10	107	105	10.8 ± 0.4
	0.04	9	97	108	13.2 ± 0.9
	0.10	8	121	146	13.5 ± 0.6
	0.50	5	117	159	18.1 ± 1.2 ($P<0.001$)

*Taken from (*1*).

†Male rats were castrated at 43 days of age and hypophysectomized at 44 days; 24 hours later, a regimen of single daily intraperitoneal injections was initiated for 10 days. Animals were autopsied on the day following the last injection.

‡Mean ± standard error.

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Table 2.—Effect of interstitial cell-stimulating hormone (ICSH) alone and in combination with bovine growth hormone on weight of ventral prostate in hypophysectomized rats and mice*

Animals	Treatment	Daily dose (μg)	Number of animals	Body weight: Onset (g)	Body weight: Autopsy (g)	Ventral prostate (mg)
Sprague-Dawley rats‡	Saline	0	6	44	42	6. 2 ± 0. 7†
	Bovine growth hormone (BGH)	2	8	50	58	8. 7 ± 0. 6
	ICSH	5	7	49	54	15. 1 ± 0. 9
	ICSH + BGH	5 + 2	7	49	58	22. 3 ± 1. 9
C3H mice§	Saline	0	11	21	18	1. 4 ± 0. 2
	BGH	100	4	21	23	1. 6 ± 0. 3
	ICSH	100	5	21	19	3. 8 ± 0. 4
	ICSH + BGH	100 + 100	5	22	25	9. 2 ± 0. 4

*Taken from (*2*, *3*).

†Mean ± standard error.

‡Single daily injections were given subcutaneously for a period of 4 days beginning at 23 days (hypophysectomy at 21 days of age) of age, with autopsy at 27 days.

§Mice hypothysectomized at 42 days of age; single subcutaneous injections begun on day of operation and continued for 30 days.

In 1927 Smith (*5*) demonstrated unequivocally the existence of a principle or principles in the pituitary gland, responsible for the physiological functioning of the reproductive organs in female and male animals. Four years later, Fevold, Hisaw, and Leonard (*6*) postulated that there were two factors concerned in these physiological processes, which they designated follicle-stimulating hormone (FSH), and luteinizing hormone (LH). We prefer to employ the term ICSH for the latter.

In 1940, two groups of investigators (*7*, *8*) reported independently the preparation of ICSH in highly purified state from ovine and porcine pituitary extracts. These earlier studies provide definitive evidence for the individuality of ICSH. In 1958, a new procedure for the isolation of ovine ICSH was described (*9*), and physicochemical characterization of the hormone by newer methods of protein chemistry was reported (*10–12*).

After the isolation and partial characterization of GH from human pituitary glands (*13*, *14*) in 1956, two other laboratories (*15*, *16*) initiated efforts to obtain human ICSH in pure form and to characterize it as a protein entity. In 1960, we reported our earlier efforts (*17*) toward the purification of ICSH from human pituitaries, with certain side-fractions obtained during the isolation of human GH used as starting material. Recently, with P. G. Squire and R. N. Andersen, we were able to obtain a product with a higher biological activity than has been reported previously and a high degree of physicochemical and biological homogeneity (*18*). Table 3 outlines the procedure, with the yield and average potency from each step. The yield of the highly purified product is extremely low: 1 mg from 10 g of lyophilized glands (approximately 100 fresh pituitaries).

TABLE 3.—Yield and potency of fractions obtained from the procedure for the isolation of human interstitial cell-stimulating hormone

Fractions	Yield (mg)	Procedure	Average potency (units/mg)
A	10, 000	Lyophilized pituitaries	
AS	4, 900	CaO extract	
U	280	IRC-50 resin chromatography	37
HA	70	pH-$(NH_4)_2SO_4$ fractionation	100
CMC	7	CM-cellulose chromatography	500
ZE	1	Column zone electrophoresis	1, 500

Table 4 summarizes some of the properties of human ICSH as compared with the ovine hormone. Both molecular weights (as determined by sedimentation equilibrium) and sedimentation coefficients are virtually identical, but the isoelectric points differ markedly: that of the human hormone is located at *p*H 5.4 as compared with 7.3 for the ovine. This difference in isoelectric point was predictable from the chromatographic behavior of the two hormones in IRC-50 resin, when it was observed that the "leak point," the critical *p*H above which the biological activity is no longer absorbed by IRC-50 resin from a 0.2 M phosphate buffer, was 5.6 for human ICSH and 6.2 for the ovine hormone. The specific activity of human ICSH is about 5 times as high as that of the ovine ICSH on the basis of results of the ventral prostate test.

TABLE 4.—Some properties of ovine and human interstitial cell-stimulating hormone

Properties	Ovine	Human
Sedimentation coefficient ($S_{20,w}$)	2. 70 S	2. 71 S
Molecular weight*	25, 000	25, 000
Isoelectric point	7. 3	5. 4
Leak point†	6. 2	5. 6
Specific activity	300	1, 500

*Determined by sedimentation equilibrium.
†Amberlite IRC-50 resin equilibrated with 0.2 M potassium phosphate buffer.

Preliminary studies, with U. Gröschel, on the carbohydrate moiety (*19*) of human ICSH indicate that it contains the following carbohydrates (mg/100 mg protein): hexose, 1.1; fucose, 0.4; hexosamine, 2.0; and sialic acid, 1.0. These values are different from those obtained with the ovine hormone; hexose, 3.2; fucose, 0.7; hexosamine, 6.3; and sialic acid, 0.8.

The amino acid contents of human and ovine ICSH are given in table 5. With the exception of proline, alanine, and tyrosine, the amino acid compositions of ICSH from ovine and human glands are remarkably similar; both have a high cystine content. No terminal residue analyses were made, and hence, it is not known whether the hormone protein is composed of a single polypeptide chain or multiple chains.

In immunological studies with N. R. Moudgal, we demonstrated that, like ovine ICSH (*20*) and human GH (*21*), human ICSH is a potent antigen (*22, 23*). The presence in rabbit antiserum of precipitating

Table 5.—Amino acid composition* of ovine and human interstitial cell-stimulating hormone

Amino acids	Ovine	Human
Lysine	9	10
Histidine	5	5
Arginine	10	9
Aspartic acid	10	12
Threonine	15	15
Serine	13	13
Glutamic acid	13	16
Proline	26	*16*
Glycine	10	11
Alanine	14	*9*
Cystine	8	9
Valine	13	13
Methionine	5	4
Isoleucine	6	5
Leucine	13	15
Tyrosine	6	*9*
Phenylalanine	12	14

*The hormone protein (2–3 mg) was dissolved in 0.5 ml of glass-distilled constant-boiling HCl and hydrolyzed for 24 hours at 110° C in a sealed, evacuated tube. The amino acid content in the hydrolysate was analyzed by the chromatographic method with a Spinco Model MS Amino Acid Analyzer. These preliminary data are unpublished results of C. H. Li and J. S. Dixon. [Residues per mole (25,000 g) of the hormone.]

antibodies specific to human ICSH was demonstrated by the qualitative ring test, double diffusion on Ouchterlony agar-gel plates, and immunoelectrophoresis. Table 6 presents results concerning the capacity of the rabbit antiserum to inhibit or neutralize effectively the activity of human ICSH in hypophysectomized male rats. Moreover, a total dose of 1.0 ml of the antiserum given over a period of 4 days effectively neutralizes the biological activity of human chorionic gonadotropin (HCG). This is particularly significant, since HCG possesses ICSH-like activity, and is also derived from a human source.

Finally, I should like to conclude this discussion on human ICSH with some details of our recent experiments on the *ovine* ICSH, results of which may point a way for future investigations of the human hormone when it becomes more readily available. It may be recalled that we have noted earlier the low species specificity of ovine ICSH as indicated by various immunochemical means (*20*). For instance, rabbit antiserum to ovine ICSH inhibits the activity not only of ovine ICSH but also of crude extracts of whale, pig, and rat pituitaries (table 7). Injections of the antiserum to normal rats inhibited the endogenous ICSH activity. Table 8 gives some preliminary data: When a daily dose of 0.5 ml of antiserum was administered intraperitoneally for 12 days into male rats (Long-Evans strain), 40 days old, the development and growth of the prostate gland were completely inhibited. There is no doubt that the production of androgens in these growing male rats was halted by the antiserum. It would be of great interest to obtain a large quantity of antiserum to human ICSH and test it in man. Needless to say, the essential requirement for such studies is that the antigen be obtained in the highest purity.

Table 6.—Effect of rabbit antiserum to human interstitial cell-stimulating hormone on the biological activity of gonadotropin preparations as assayed in immature hypophysectomized male rats*

Group	Number of rats per group	Material assayed	Total dose in 4 days (μg+ml)	Weight of ventral prostate (mg)	Statistical comparison of groups
1	9	Uninjected controls	0	7.0 ± 0.14†	
2	15	Human ICSH	8+0	18.1 ± 0.49	
3	7	Human ICSH + antiserum	8+1	7.5 ± 0.30	2, 3; $P < 0.001$‡
4	6	HCG§	1+0	15.5 ± 1.50	
5	5	HCG + antiserum	1+1	10.0 ± 0.83	4, 5; $P < 0.01$

*Taken from (*22*).

†Mean ± standard error

‡Values calculated according to Fisher's *t* test

§Human chorionic gonadotropin.

Table 7.—Effect of rabbit antiserum (A/S) to ovine interstitial cell-stimulating hormone on the biological potency of rat pituitary extract (RPE) in hypophysectomized male rats[*]

Treatment[†]	Total dose in 4 days (mg + ml)	Number of rats	Ventral prostate (mg)
Control	0 + 0	9	7. 0 ± 0. 1[‡]
ICSH	0. 008 + 0	27	14. 5 ± 0. 2
ICSH + A/S	0. 008 + 1	9	7. 6 ± 0. 1
RPE	4 + 0	5	21. 6 ± 0. 4
RPE + A/S	4 + 1	6	7. 8 + 0. 9

[*] Taken from (*20*).

[†] Single daily injections (subcutaneously) were given for 4 days, beginning at 23 days (hypophysectomized at 21 days of age, Long-Evans strain) of age, with autopsy at 27 days.

[‡] Mean ± standard error.

Table 8.—Effect of rabbit antiserum to ovine interstitial cell-stimulating hormone on the weights of ventral prostate in the rat

Group[*]	Number of rats	Body weight Onset (g)	 Autopsy (g)	Ventral prostate[†] (mg)
Onset controls	4	115	—	64. 2 (56. 7–69. 3)
NRS-treated	4	118	191	142. 1 (122. 8–168. 0)
Antiserum-treated	4	118	181	18. 2 (14. 4–24. 6)
Uninjected autopsy controls	5	118	182	125. 9 (100. 0–152. 3)

[*] Male rats of Long-Evans strain were injected intraperitoneally once daily with 0.5 ml normal rabbit serum (NRS) or rabbit antiserum to ovine ICSH at 40 days of age for 12 days; autopsy was performed 24 hours after the last injection.

[†] Mean (range).

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DISCUSSION

Dr. Bishop: Dr. Li, would you get the same indications of cross reactivity or lack of cross reactivity when these products are tested by gel-diffusion methods and by immunoelectrophoresis? Is there a parallel between the serological reactions and the bioassays?

Dr. Li: Yes, the bioassays and the immunological tests are comparable.

Dr. Williams-Ashman: I wonder if Dr. Li could say something about the carbohydrates associated with highly purified human ICSH.

Dr. Li: The nature and quantity of the carbohydrate moieties in ovine and human ICSH are different. Modification of these carbohydrates in ovine ICSH by periodate oxidation did not cause complete destruction of hormonal activity.

Dr. Furth: Antihormone therapy presents a problem. The antibodies of foreign species are themselves antigenic, and therefore their continued injection would be ineffective and would involve the hazard of anaphylactic reaction. You would have to devise a method of autoimmunization to the growth hormone. There have been many attempts to degrade antibodies in order to render them nonantigenic and thereby permit their prolonged use—thus far, with no success. To do this would be an even greater discovery than the isolation of pituitary hormones you accomplished.

PITUITARY FACTORS INFLUENCING GROWTH OF THE PROSTATE[1, 2]

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NORMAL growth of the prostate and other accessory sex glands of the normal male mammal has been shown repeatedly to depend on sustained pituitary function. The accepted and easily demonstrated mechanism of this dependence is an indirect one, mediated through the testis. Administration of proper gonadotropin preparations to hypophysectomized animals produces a marked stimulus to the accessory sex glands to increase in size and recover functional capacity. Administration of androgens has a similar effect. Pituitary stimulation of the adrenals may stimulate prostatic growth, but the evidence remains equivocal (*1*, *2*).

Demonstration of an end organ effect of pituitary secretions on prostate growth has not been so certainly or readily achieved as the demonstration of the indirect effect mediated through the testis. Nevertheless, considerable evidence to support this concept of an effect of the pituitary on the prostate, independent of other endocrine organs, has accumulated since the observation of Huggins and Russell (*3*) concerning the apparent increase in atrophy of the prostate after hypophysectomy in the dog. It is my purpose to present a summary of this evidence, emphasizing some material from our own experience.

EXPERIMENTAL EVIDENCE

Evidence suggesting an independent effect of the pituitary on the prostate has been derived principally from studies on dogs and rats. Gravimetric, histologic, chemical, and functional indicators of prostatic status have been utilized in these studies. The concept of a direct pituitary effect on the prostate is based on comparison of these indicators in hypophysectomized or gonadectomized animals that are untreated

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] This work was supported in part by the Lucy and Edwin Kretchmer Fund of Northwestern University Medical School and by grant C-05536 from the National Cancer Institute, National Institutes of Health, Public Health Service.

or which have been given an androgen alone or supplemented with a pituitary derivative.

Degree of Atrophy After Hypophysectomy

Huggins and Russell (*3*) first noted that the prostate of the hypophysectomized dog showed a greater degree of epithelial atrophy on histologic examination than the prostate of a comparable gonadectomized animal. Lostroh and Li (*4*) subsequently made a similar observation on the ventral prostate of rats of the Long-Evans strain.

Greater loss of prostatic weight has been noted in rats subjected to hypophysectomy than in those subjected only to castration (*4, 5*). Our over-all experience tends to confirm this observation.

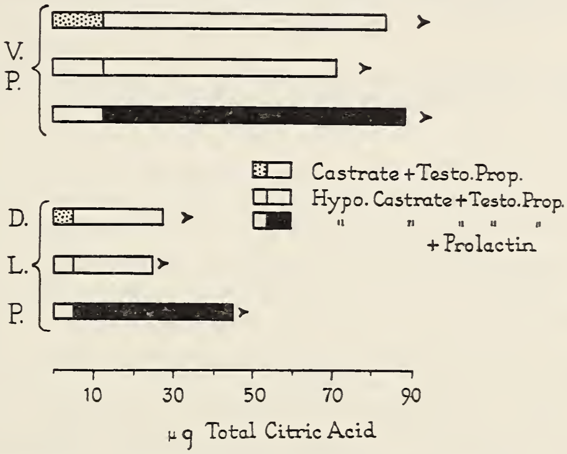
The marked decrease in concentration of acid and alkaline phosphatase in the prostate of the hypophysectomized dog as compared to the gonadectomized animal provides enzymatic evidence of increased atrophy of this organ in the hypophysectomized dog (*3*). Vanderlaan made similar observations with regard to alkaline phosphatase in the rat (*5*). In limited studies in our laboratory with Sprague-Dawley rats, hypophysectomy has not resulted in any greater change in citric acid or fructose content or concentration of the prostate than has castration.

Response to Androgen After Hypophysectomy

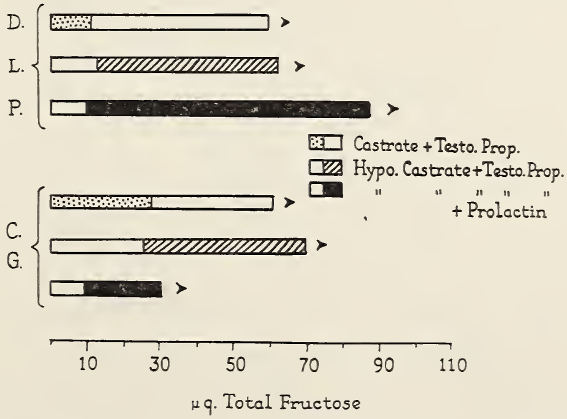
At about the time we had confirmed our original observations, Vanderlaan (*6*) reported in an abstract that administration of small amounts of testosterone propionate resulted in less growth of the ventral prostate in hypophysectomized-castrated rats than in rats subjected only to castration. Our findings (*7*) confirmed these, although the combined weight of the dorsolateral and ventral prostate of the Sprague-Dawley rat was utilized as the indicator of prostatic response to androgen.

This diminished response to testosterone propionate, though most readily demonstrated with large amounts of this androgen (0.5 to 4 mg every other day) has been observed repeatedly in our laboratory in the hypophysectomized-castrated Sprague-Dawley rat with administration of amounts of testosterone propionate varying from 0.1 to 4 mg every other day for 12 to 14 days. Force feeding the hypophysectomized rats and severely restricting the dietary intake of the castrated rats with intact pituitaries has not equalized the response of these groups. In our experience, the reduction in response to androgen was slightly greater in the ventral than the dorsal lateral lobe of the prostate of the hypophysectomized-castrated rat. In Vanderlaan's studies (*5*) the smallest amount of testosterone propionate employed, 3 μg daily for 9 days, produced the greatest percentage difference in response of the ventral prostate of hypophysectomized and hypophysectomized-castrated rats. In contrast to these results, no failure of response to testosterone propionate has been observed in hypophysectomized rats of the Long-Evans strain (*8*).

Administration of 0.1 mg of testosterone propionate every other day for 12 days to castrated and hypophysectomized-castrated Sprague-Dawley rats produced no definite difference in total citric acid or concentration of citric acid in the ventral or dorsolateral prostates (text-fig. 1) (*9*). In these animals the total amount and concentration of fructose were comparable in both groups (text-fig. 2). The limited ob-



TEXT-FIGURE 1.—Final citric acid content and concentration in ventral prostate (V.P.) and dorsolateral prostate (D.L.P.) of young adult Sprague-Dawley rats orchiectomized 3 weeks and hypophysectomized 2 weeks at the time injection of 0.1 mg testosterone propionate subcutaneously every other day for 12 days was initiated. Prolactin, 2.5 mg (Panlitar, Armour & Co., Chicago, Ill.) was given subcutaneously daily. Autopsy was performed 2 days after final testosterone injection. > Indicates magnitude of standard error. Lower portion of each bar represents concentration in mg per 100 g of tissue; the total bar represents μg total citric acid content.



TEXT-FIGURE 2.—Final fructose content and concentration in dorsolateral prostate (D.L.P.) and coagulating gland (C.G.) of rats described in text-figure 1. > Indicates magnitude of standard error. Lower portion of bar represents concentration in mg per 100 g of tissue.

servations of Mann and Parsons (*10*), suggesting the possibility that the fructose formation of the prostate and glandula vesicularis in response to testosterone is less in the hypophysectomized than in the castrated rabbit, require confirmation.

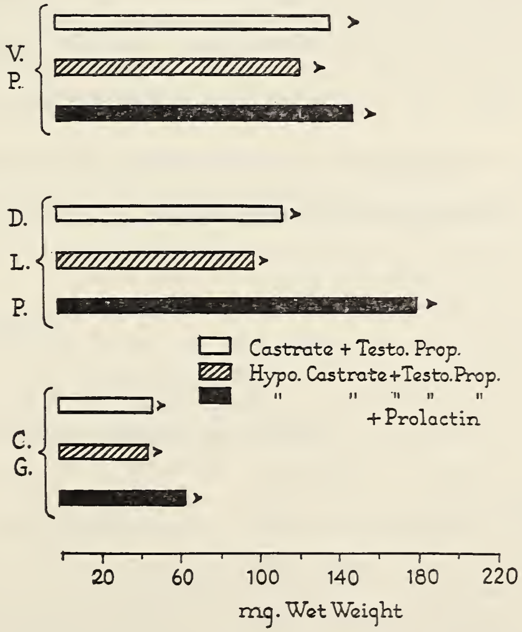
Restoration of the secretory ability of the prostate of the hypophysectomized dogs by androgen administration has been demonstrated by Goodwin *et al.* (*11*). No attempt has been made, however, to quantify the response to exogenous androgen before and after hypophysectomy.

Response to Androgen and/or Pituitary Replacement Following Hypophysectomy

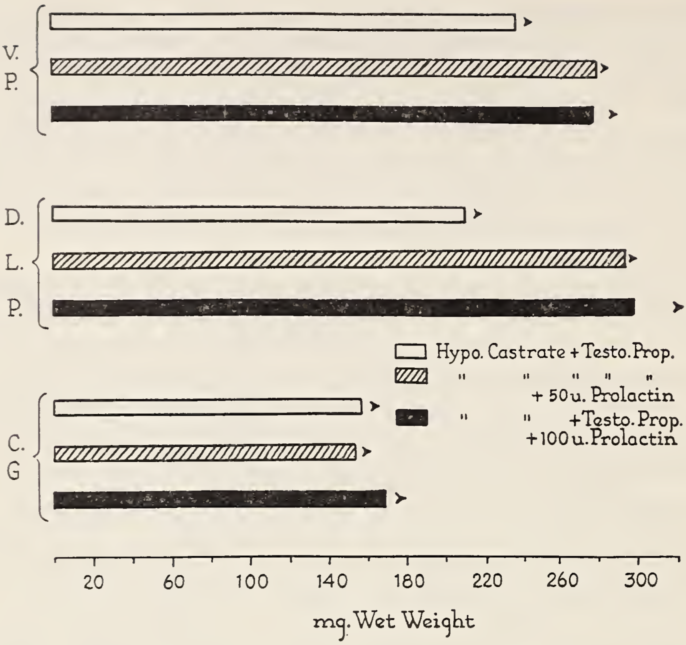
A number of investigators have demonstrated that the simultaneous administration of an androgen and a pituitary extract to hypophysectomized-castrated male Sprague-Dawley rats results in a greater prostatic weight than administration of an androgen alone. In our initial observations (*7*) simultaneous administration of adrenocorticotropin, cortisone, or gonadotropin (Antuitrin "S," Parke, Davis & Co., Detroit, Mich.) and testosterone propionate revealed no definite evidence of a synergistic prostatic effect between these substances. On the other hand, administration of a commercial prolactin preparation (Luteotrophin, E. R. Squibb & Sons, New York, N.Y.) with the testosterone propionate resulted in a definitely greater prostatic weight than with testosterone propionate alone. Thyroxin given with testosterone propionate caused a questionable augmentation of the effect of the latter. The apparent synergism between prolactin and exogenous androgen in the hypophysectomized animals persisted even in the face of loss of body weight. In a number of other experiments, a few of which are summarized in this report, we have always been able to duplicate these results.

In the studies cited, the total weight of the ventral and dorsolateral prostate was utilized as the indicator of prostatic response. Although great care was not taken in separating the ventral from the dorsolateral lobes, the dorsolateral seemed to demonstrate the more marked response to the pituitary supplement. Others investigating this problem have utilized the weight and/or histologic changes in the ventral prostate as the primary indicator of prostatic response. The coagulating gland or anterior prostate has been employed as an ancillary indicator, but the dorsolateral prostate has been excluded from consideration. Under these circumstances, simultaneous prolactin or growth hormone and testosterone propionate administration to Long-Evans rats produced no greater weight response than testosterone propionate alone (*12*). However, in the Sprague-Dawley strain, Chase *et al.* (*12*) repeatedly observed an increased weight response of the ventral prostate to simultaneous prolactin and testosterone propionate administration; although individually these observations were of questionable significance, their persistence on repetition of the experiment adds to their validity. In these experiments, a definite synergism between prolactin, growth hormone, and testosterone propionate was evident. Segaloff *et al.* (*13*) and Lostroh *et al.* (*14*) have demonstrated an apparent synergism between prolactin and ICSH-stimulated endogenous androgen in hypophysectomized Sprague-Dawley rats. The Lostroh group noted a more consistent effect with growth hormone. Huggins, Parsons, and Jensen (*15*) had previously noted an increased weight response of the ventral prostate in castrated-hypophysectomized rats of this strain given bovine growth hormone and testosterone propionate, as contrasted with those given only androgen.

Recent observations in our laboratory demonstrate the importance of considering the weight of the dorsolateral prostate in evaluating the response of the rat prostate to prolactin or other preparations (*9, 16*). In 2 studies employing hypophysectomized-castrated rats of the Sprague-Dawley strain, the relative increase in weight elicited in the dorsolateral prostate by supplementation of a standard amount of testosterone propionate with a prolactin preparation (Panlitar, Armour & Co., Chicago, Ill.) was considerably greater than in other portions of the prostate (text-figs. 3 and 4). With 0.1 mg testosterone propionate every other day for 12 days (text-fig. 3), the ventral prostate showed only a minimal response to supplementation with Panlitar. Had the ventral prostate been the only indicator employed, this prolactin preparation may have been judged to be without effect on the prostate of the hypophysectomized-castrated Sprague-Dawley rat.



TEXT-FIGURE 3.—Final weight of ventral prostate (V.P.), dorsolateral prostate (D.L.P.), and coagulating gland (C.G.) of rats described in text-figure 1. >Indicates magnitude of standard error.

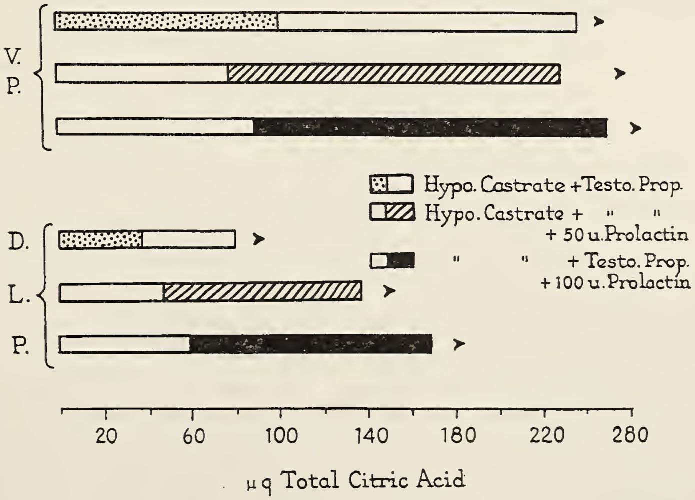


TEXT-FIGURE 4.—Final weight of ventral prostate (V.P.), dorsolateral prostate (D.L.P.), and coagulating gland (C.G.) in young adult Sprague-Dawley rats hypophysectomized 20 days at the time injections of 1.0 mg of testosterone propionate subcutaneously every other day for 12 days were initiated. Castration was performed 5 or 13 days after hypophysectomy. Prolactin (Panlitar, Armour & Co.) was given subcutaneously daily. Autopsy was performed 2 days after final testosterone injection. >Indicates magnitude of standard error.

Histologic evidence of stimulation of the rat ventral prostate was noted by Lostroh and Li (*4*) following administration of bovine growth hormone to hypophysectomized-castrated rats of the Long-Evans strain; in this experiment, bovine growth hormone administered with an active corticoid also resulted in an increase in ventral prostate weight. Prolactin alone produced no effect in either the Long-Evans or the Sprague-Dawley strain. However, histometric analysis of the ventral prostate of hypophysectomized-castrated rats of the latter strain receiving 150 μg of prolactin and 25 μg of testosterone propionate daily for 5 days revealed an increase in the proportion of glandular tissue when compared to animals receiving only 25 μg of testosterone propionate for this period (*12*). Growth hormone had no such effect.

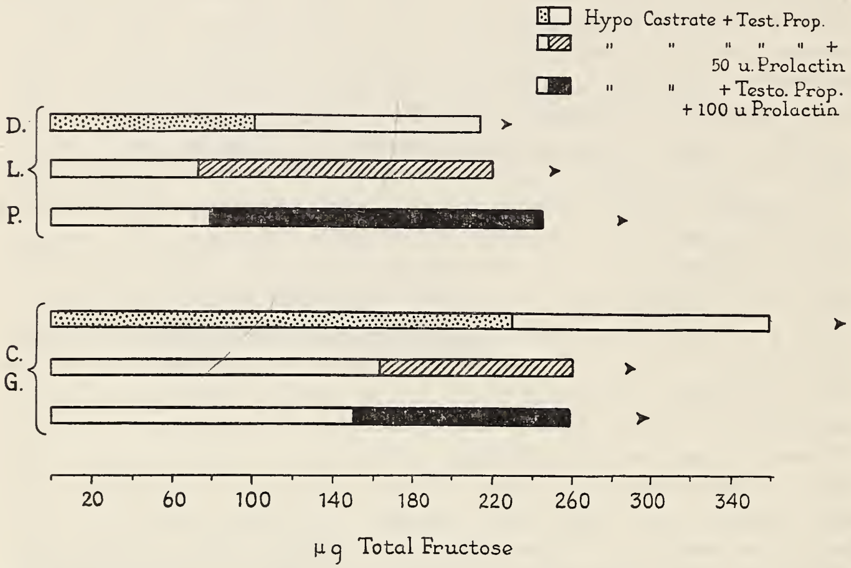
Experiments to evaluate the effect of administration of prolactin (Panlitar) on the chemical indicators of androgen activity are being carried out in our laboratory. The citric acid content of the ventral and dorsolateral prostate and the fructose content of the dorsolateral prostate and the coagulating gland have been determined in the groups of Sprague-Dawley rats previously discussed. Text-figures 1 and 2 present the data with regard to citric acid and fructose content of the accessory sex glands of the group receiving prolactin in addition to 0.1 mg testosterone propionate. The total citric acid content of the dorsolateral prostate was significantly ($P<0.01$) greater in the hypophysectomized-castrated rats receiving prolactin and testosterone propionate than in the castrated or hypophysectomized-castrated rat receiving only testosterone. However, this is a reflection of the increase in size of this organ in this group; no increase in concentration of citric acid was evident in any group. Similar observations were made concerning the fructose content of the dorsolateral prostate. A significant decrease in total fructose content of the coagulating gland in the prolactin-treated group as compared to the castrated animal receiving testosterone propionate requires confirmation.

Text-figures 5 and 6 present the data with regard to citric acid and fructose content of the accessory sex glands of the group of Sprague-Dawley rats receiving 1 mg of testosterone propionate and either 50 or 100 units of Panlitar daily. The total citric acid content of the dorsolateral prostate of rats receiving prolactin and testosterone propionate was significantly ($P<0.01$) greater than that of rats receiving only testosterone propionate. Of even greater interest was an apparent increase in concentration of citric acid in both groups of prolactin-treated animals (137 and 168 mg per 100 g of tissue, wet weight) as compared to animals receiving testosterone propionate only (80 mg per 100 g of tissue). The wide variation in concentration observed makes the validity of these figures questionable. If these results were confirmed on repetition, they would be further substantial evidence for a direct or indirect synergism between some component of the prolactin preparation employed and



TEXT-FIGURE 5.—Final citric acid content and concentration in ventral prostate (V.P.) and dorsolateral prostate (D.L.P.) of rats described in text-figure 4. The lower portion of each bar represents concentration in mg per 100 g of tissue; the total bar represents μg total citric acid content. >Indicates magnitude of standard error.

testosterone propionate in promoting prostatic growth in the hypophysectomized-castrated Sprague-Dawley rat. Again in these experiments there was an apparent but uncertain reduction in total fructose content of the coagulating gland.



TEXT-FIGURE 6.—Final fructose content and concentration in dorsolateral prostate (D.L.P.) and coagulating gland (C.G.) of rats described in text-figure 4. The lower portion of each bar represents concentration in mg per 100 g of tissue. >Indicates magnitude of standard error.

DISCUSSION

The evidence presented supports the concept of a pituitary effect on prostatic growth that is not mediated through the testes. Since observations providing this support have been confined to 2 strains of rats and the dog, we cannot assume that this phenomenon is widespread in the animal kingdom. However, the evidence for this pituitary effect on the prostate has been derived in studies utilizing histologic, gravimetric, and chemical indicators of degree of prostatic growth under conditions of deficiency and replacement of hypophyseal secretions. The effect of hypophysectomy on the function of the prostate has not been systematically evaluated.

Reservations regarding the end organ effect of pituitary secretion on the prostate stem from the reported failure of hypophysectomized rats of the Long-Evans strain to show consistent evidence of a synergistic response of the ventral prostate to simultaneous administration of exogenous androgen and prolactin (*12*) or to simultaneous administration of interstitial cell-stimulating hormone and prolactin (*14*). Observations in Sprague-Dawley rats indicate that the tendency to ignore evaluation of the dorsolateral prostate in assessing prostatic response may have been a critical factor in these negative results. Furthermore, evidence of a pituitary effect on the prostate of rats of the Long-Evans strain has accumulated. The degree of prostatic atrophy is greater after hypophysectomy than following castration in this strain. Histologic and gravimetric evidence suggests stimulation of this atrophic prostate can be achieved by bovine growth hormone given with an active corticoid (*4*). Administration of growth hormone also resulted in an augmentation of the prostate response of hypophysectomized Long-Evans rats to subcutaneous injections of interstitial cell-stimulating hormone. These facts support the concept of a pituitary effect on the prostate in this strain of rats.

The exact nature of the pituitary fraction affecting the prostate and its mechanism of action is uncertain. A synergistic effect between testosterone propionate and the Armour brand of ovine prolactin, Panlitar, has been observed consistently in our laboratory. In the absence of testosterone, prolactin has never enhanced the weight of the prostate or given any evidence of independent prostatic stimulation such as has been reported for growth hormone. Others (*4*) failed to note evidence of stimulation of the ventral prostate of hypophysectomized Long-Evans rats even when 5 mg of sheep lactogenic hormone was administered. Most of our observations have been made in Sprague-Dawley rats receiving large doses, *e.g.*, 2.5 mg of prolactin daily, but even with much smaller amounts we have observed enhancement of the prostatic response to androgen. To my knowledge, no evidence contradicting the effectiveness of growth hormone as an androgen synergist has been convincingly presented. There is a reasonable possibility that both prolactin and growth hormone, if they are definitely separate entities, may possess this characteristic. Some evidence suggests that they may even enhance the activity of each other and that their mode of action may differ (*12*). Of prime importance is the fact that the evidence indicates prostatic growth of a hypophysectomized-castrated rat can be made to approximate that of a castrated rat with pituitary intact, if the androgen administered to both is supplemented in the former by a substance extracted from whole pituitary.

The mechanism of this increased response to androgen associated with administration of a pituitary derivative is not clear. With varying circumstances any of the accessory organs of the rat, including the seminal vesicle (*17*) and coagulating gland, may show gravimetric evidence of synergism between an administered pituitary fraction and androgen. in our experience with prolactin preparations, some slight increase in weight in all accessory sex glands usually occurs under these circumstances, as does a slight increase in body weight. These observations suggest a nonspecific anabolic response to the pituitary substance administered in conjunction with the androgen to the hypophysectomized-castrated rats. However, the magnitude of the weight response in the dorsolateral prostate noted in the experiments reported (text-figs. 3 and 4) far exceeds any other organ or body weight increase observed. This suggests a more specific effect of the administered pituitary extract, Panlitar, in these experiments. However, the evidence is still insufficient to be certain that this response is a direct end organ effect.

The selective response of the dorsolateral prostate noted is not without precedent. This portion of the rat's prostate differs from the other prostatic tissue in other regards, such as its ability to concentrate zinc under stimulation with androgen (*18*) and a marked synergism between estrogen and androgen in accumulation of citric acid (*19*). If the increase noted in citric acid concentration as well as total citric acid in the dorsolateral prostate (text-fig. 5) can be confirmed, this would be further evidence of a unique response of this portion of the prostate to an administered hormone.

SUMMARY AND CONCLUSIONS

The evidence of a pituitary effect on the prostate of the rat and dog, not mediated through the testis, is reviewed. With the histologic, gravimetric, and chemical indicators of prostatic status employed, the observations made of animals studied after pituitary ablation, hypophysectomy followed by replacement with androgen, and hypophysectomy followed by replacement with androgen and a pituitary extract, support the concept of an independent pituitary effect on the prostate. The pituitary substance or substances primarily responsible for this effect are not definitely established, although it seems likely that both prolactin and growth hormone as presently identified increase the effect of administered androgen in hypophysectomized rats. The exact mechanism of this pituitary end organ effect remains uncertain.

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DISCUSSION

Dr. Li: Dr. Grayhack did a beautiful job in using every part of the prostate to clarify the problem. We did publish some work on prolactin effect—and the lack of this effect. Of course we could not repeat this consistently, perhaps because we employed ventral prostate. I would like to ask Dr. Grayhack whether he has analyzed citric acid and fructose contents in the dorsal prostate when bovine growth hormone was injected into the experimental animals.

Dr. Grayhack: No, we have not.

Dr. Hollander: I should like to ask Dr. Grayhack if he has done any experiments to study the effects of castration plus adrenalectomy in comparison with hypophysectomy.

Dr. Grayhack: No. This is apparently going to be subject for discussion later ("Hormonal Factors in the Adrenal-Dependent Growth of the Rat Ventral Prostate"). We gave ACTH without evidence of synergism, and in all these experiments we measured adrenal weight, to see if there was any gravimetric evidence of adrenal stimulation.

Dr. Li: I think I can answer this question partially. Dr. Lostroh in our laboratory has injected bovine growth hormone in hypophysectomized-castrated-adrenalectomized rats. The ventral prostate weight and the histologic changes have been observed after administration of bovine growth hormone.

Dr. Rosenkrantz: We recently published some data comparing adrenalectomized-castrated, adrenalectomized-hypophysectomized, and castrated Sprague-Dawley rats. These were young rats. We measured the wet weights of the ventral prostate, and in addition we measured the total endogenous dehydrogenases as reflected in the deposition of formazan by triphenyltetrazolium. There were no significant differences between hypophysectomized and castrated rats in the ventral prostate weights or in the total dehydrogenase levels. Of course the values decreased markedly as compared to intact animals. There was no contribution to further inhibition by adrenalectomy. Primarily the effect of hypophysectomy was seen in adrenalectomized-hypophysectomized rats. In the group that was only adrenalectomized, there was a stimulation of both the weight and the total dehydrogenase.

NUTRITION AND ACCESSORY SEX GLANDS[1, 2]

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The functional integrity of the endocrine system is dependent upon adequate nutrition. Not only does nutrition influence the synthesis and release of hormones, but hormones in turn, through their regulation of metabolism, influence nutritional requirements (*1*, *2*). Experimental and clinical studies have shown that chronic and acute starvation, caloric restriction, vitamin and mineral deficiencies, and the quality and quantity of protein alter endocrine gland function. In addition, while frequently overlooked, the effects of excesses and overnutrition can be as deleterious as undernutrition.

In reproduction, the delicate balance between the hypophysis and the testis can be altered by inadequate nutrition (*3*, *4*). Deficiencies in the diet may alter testis function as to spermatogenesis, steroid production, or both. Furthermore, the promptness with which the male accessory organs react makes them very useful in a study of nutritional deficiencies that influence the testis or the hypophyseal-gonadal axis.

HYPOPHYSIS AND DIET

Studies involving acute and chronic starvation have shown that gonadal hypofunction during inanition is primarily due to diminished levels of circulating gonadotropins. Because of the similarity to changes following hypophysectomy, the endocrine response to inanition has been referred to as "pseudohypophysectomy."

Human reproductive disturbances associated with undernutrition have implicated the hypophysis as urinary gonadotropins decrease (*5*). Furthermore, hypophyseal damage may result from severe restriction of food at puberty; continued hypophyseal hypofunction has been observed despite corrections of the nutritional deficiency. In experimental animals subjected to undernutrition, hypophyseal gonadotropin content has been reported as decreased, increased, or unchanged (*4*).

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Hypophyseal hormones are protein in nature, and the amino acids for protein synthesis must be drawn from body sources. The body nitrogen source is obviously influenced by dietary protein. Thus, if dietary protein is increased to 90 percent, hypophyseal gonadotropin content increases, whereas a diet lacking in protein causes a decrease in gonadotropin content. Srebnick and Nelson (*6*) have shown that follicle-stimulating hormone and luteinizing hormone are reduced by protein-free diets.

Vitamin deficiencies influence hypophyseal gonadotropin content. A lack of vitamin A, E, or B will increase hormone content and a B_6 deficiency will augment follicle-stimulating hormone tenfold.

TESTIS AND DIET

The two basic functions of the male gonads are to produce gametes and secrete steroids. Spermatogenic activity can be estimated from testis morphology and examination of semen samples. Steroid secretion can be estimated from urinary steroid levels, accessory sex gland weights, and chemical composition of secretions.

Testis maturation is prevented by undernutrition, subnormal dietary protein levels, amino acid deficiencies, caloric restriction, lack of essential fatty acids, and certain vitamin omissions. Undernutrition in 700 boys, 7 to 16 years of age, markedly increased genital infantilism and cryptorchidism (*7*).

In the bull, normal maturation reveals that androgen secretion precedes spermatogenesis. Restriction of food intake to one half has a marked delaying action on androgen secretion and a lesser effect on spermatogenesis (*8*).

The reproductive organs of the adult are more resistant to dietary inadequacies than those of the immature animal. Loss of Leydig cell function is followed some time later by a reduction in spermatogenesis in animals and man, in association with chronic malnutrition. However, both functions of the testis are not always influenced, *e.g.*, ethionine causes tubular atrophy and Leydig cell hyperplasia (*9*, *10*).

ACCESSORY REPRODUCTIVE ORGANS AND DIET

Seminal vesicles and prostate glands are quickly influenced by undernutrition and assume a castrate-like size (*4*). Futhermore, Pazos and Huggins (*11*) noted a sharp reduction in prostatic fluid volume in the dog completely deprived of food. Withholding food from adult mice for 3 days reduced seminal vesicle weight and water and protein concentrations; glycogen was increased (table 1). Restricting food intake to half normal in maturing bull calves had a markedly delaying effect on the onset of accessory sex gland secretion (*8*).

TABLE 1.—Effect of starvation and androgen on adult mouse seminal vesicle

Treatment	Number of mice	Organ weight (mg)	Water concentration (%)	Protein		Glycogen (%)
				Percent	Total	
Control	16	51	77. 0	82. 3	9. 8	0. 20
Starved for 3 days	16	39	72. 6	79. 0	8. 7	0. 35
Control + Tp*	12	57	73. 0	80. 4	12. 1	0. 50
Starved + Tp	12	56	76. 5	79. 3	10. 3	0. 41

* Tp = testosterone propionate; 1 subcutaneous injection of 0.25 mg Tp.

When 22-day-old male rats were fed a 20 percent casein diet for 30 days, weight of the seminal vesicle increased from 9 to 88 mg and weight of the ventral prostate from 7 to 61 mg. Reducing dietary protein to 6 percent permitted a twofold increase in accessory gland weight, whereas further protein restrictions prevented growth of the glands (*12*).

Not only is the level of dietary protein an influence on accessory gland development but also the quality of protein fed. Substituting wheat gluten and peanut flour at the 20 percent level in place of casein in the diet limited seminal vesicle weight increase to less than 100 percent (*12*).

Accessory gland secretions contain fructose and citric acid, which serve as excellent indicators of androgen secretion (*3*). The fructose in the semen may depend on tissue glycogen as a precursor, but histological studies have not revealed glycogen in the accessory sex glands of many mammals. Nevertheless, glycogen, as estimated by the anthrone procedure, is present in the seminal vesicles of mice and at greater concentrations in the immature than in the mature mouse. Feeding a protein-free diet retained glycogen at a high level but prevented seminal vesicle growth. In contrast, water and protein concentrations were reduced by protein depletion. β-Glucuronidase has been correlated with growth processes, and Knobil (*13*) noted an increase in this enzyme in the rat seminal vesicle following steroid administration but no change with castration. Nevertheless, protein depletion influences the enzyme in immature normal and castrated rats. The castrated rat fed 20 percent casein had a seminal vesicle weight of 14.5 mg and a β-glucuronidase activity of 1957 units per g; protein-free feeding reduced seminal vesicle weight to 6.8 mg and enzyme activity to 798 units per g (table 2). A nutritional effect directly on the accessory organs is suggested.

TABLE 2.—Seminal vesicle weight and β-glucuronidase activity in immature male rats deprived of dietary protein

Diet	Days fed	Number of rats	Seminal vesicle weight (mg)	β-Glucuronidase	
				Units per g	Total
Initial	Controls	10	13. 2	2,585	34
20% Casein	24	10	14. 5	1,957	29
0% Casein	24	8	6. 8	798	5

Withholding dietary protein from an immature rat for 30 days, during which time maturation will occur in the fully fed animal, does not impose a permanent damage. Refeeding adequate protein permits recovery of spermatozoa and a more gradual return of the accessory organ size (*12*).

Protein anabolic tendencies are greater in the tissues of young growing animals, and their bodies are more dependent on dietary protein level and protein quality for the maintenance of the metabolic pool of nitrogen than those of adult animals. Furthermore, the adult can shift protein internally when dietary protein sources are reduced. Thus, it is not surprising that removal of protein from the diet for 30 days had little effect on adult rat testis weight but reduced seminal vesicle weight from 1142 to 689 mg; extending the depletion period augmented the organ weight loss (*4*).

Adequate dietary protein can not maintain the accessory sex organs if the diet is calorie-deficient. Thus, a decrease in seminal vesicle weight could be related to a decrease in dietary calories while protein levels are held constant (*14*). However, the accessory weight loss imposed by caloric restriction could be slowed by an increase of dietary protein (*15*). In our studies, adult male rats fed a protein-free diet daily of 100 or 200 calories per kg of body weight had seminal vesicle weights of 250 mg, as compared with 475 mg in rats fed 20 percent protein. Increasing the protein level of the diet to 40 percent but restricting intake to 100 calories resulted in seminal vesicle weights of 353 mg. Despite organ weight changes, no significant influence on β-glucuronidase was noted.

Calorie restriction has also been examined in adult rats in relationship to protein quality. Adult male rats were fed 100 calories per kg daily for 20 days. The diet contained one of the four proteins tested, and seminal vesicle weight was distinctly less when gelatin or lactalbumin was fed than when casein or wheat gluten was the dietary protein source (table 3).

Subnormal seminal vesicle weights have been noted in tumor-bearing animals (*16*), possibly due to reduced androgen production. We have also

TABLE 3.—Seminal vesicle weight as influenced by diet, Walker 256 tumor, and testosterone propionate in adult rats

Diet* (18%)	Testosterone propionate (mg)	Number of rats	Seminal vesicle weight (mg/100 g) Normal rats	Rats with tumors
Casein	0	8	216	125
	0. 5†	7	332	241
Lactalbumin	0	6	119	106
	0. 5	6	215	195
Wheat gluten	0	7	205	109
	0. 5	8	356	184
Gelatin	0	9	142	100
	0. 5	7	290	216

*One hundred calories per kg per rat daily.
†Daily for 20 days

noted that a Walker 256 tumor will reduce seminal vesicle weight in adult male rats fed 100 calories per day. The tumor-depleting effects exceeded those of caloric restriction alone. Furthermore, quality of protein did not modify the tumor-depleting action (table 3).

Atrophy of the adult male reproductive system can be induced by estrogen administration, and over a 20-day period dietary protein levels of 18 percent or 0 percent do not modify the results. After discontinuance of the hormone and the feeding of an 18 percent casein diet to all animals, seminal vesicle weight recovery was clearly less rapid in rats fed a protein-free diet while receiving hormone.

Reproductive system dysfunction, as judged by failure of sperm formation or atrophy of the secondary sex organs, has been observed in deprivation of thiamine, riboflavine, pyridoxine, calcium pantothenate, biotin, and vitamins A and E. One must distinguish, however, between effects of inanition and a specific vitamin effect and also consider species differences. Thus, a vitamin E deficiency is species-limited and does not involve androgen production by the testis. Vitamin A deficiency in bulls before breeding age will prevent breeding; adult bulls may exhibit a lower quality semen (*17*). Vitamin A deficiency induces metaplastic keratinization of the epithelial lining of the male secondary sex glands, and thus semen may be influenced. Depletion of vitamins in the B complex, especially thiamine, specifically influences the Leydig cells and prevents growth of the accessory sex organs (*3*).

INFLUENCE OF NUTRITION ON THE HORMONE RESPONSIVENESS OF MALE SEX ACCESSORY GLANDS

Atrophy of the seminal vesicle and ventral prostate due to inanition is suggested as due to a lack of androgen production rather than a failure of organ response. Despite chronic underfeeding, administration of gonadotropin is known to increase the weight of these organs. Furthermore, chorionic gonadotropin restores fructose and citric acid levels despite a vitamin B deficiency. Nevertheless, an influence of inadequate nutrition on accessory gland sex organ weight was observed in immature mice in which the response to pregnant mare serum ganadotropin decreased when the feeding of a protein-free diet was extended from 10 to 20 days (*12*).

The accessory reproductive organs respond to direct stimulation despite an inadequate food intake. In fact, in the starved dog, androgen restored prostatic fluid volume, increased acid phosphatase, and induced growth of the organ (*11*). In vitamin B deficiency, testosterone restored fructose and citric acid levels to normal in rat seminal vesicles despite the need for thiamine in carbohydrate metabolism (*18*). Inadequate food intake may, however, cause a subnormal seminal vesicle or prostate weight response to androgen (*19–21*). Using fructose and citric acid in the semen as indicators, Mann *et al.* (*22*) have shown that underfeeding of young bull calves decreased their responsiveness to testosterone. Lack of folic acid reduced the effect of estrogen on the prostate (*23*), whereas supplementation of folic acid improved testosterone action (*20*).

Protein depletion reduces the supply of amino acids to the metabolic pool, and thus amino acid availability for accessory gland protein synthesis, as well as the synthesis of growth hormone, may be reduced. Growth hormone appears to enhance the response of the seminal vesicles to androgen (*24*). Obviously, the degree of protein depletion will influence the possibility of a normal or subnormal response to androgen. Thus, despite the depleting effects of a tumor on body protein stores over a 20-day period, we have not seen a lack of response to androgen or estrogen. Imposing additional demands on available protein by subjection of tumor-bearing rats to caloric restriction also did not prevent a response to androgen, but the final seminal vesicle weight was significantly less than that of calorie-restricted non-tumor-bearing rats (table 3).

If the adult male rat is fed a protein-free diet for 30 days, the protein stores of the body are depleted. To test the responsiveness of these rats to androgen, the animals were hypophysectomized and either continued on the protein-free diet or fed an 18 percent casein diet. Testosterone propionate, 0.25 mg daily, induced a significantly greater response in hypophysectomized rats fed protein than in those not fed protein, as indicated by accessory gland weights, testis, and spermatogenesis. Prior protein-depleted rats, then hypophysectomized but not thereafter given androgen, failed to reveal a diet effect on organ atrophy.

Protein depletion is more readily accomplished in the immature animal. Protein depletion for a 10-day period may be sufficient to reduce the response to androgen, depending upon the nutritional state of the animal prior to experimentation. To characterize further the need for nutrition during response to androgen, the tissues were examined for alkaline phosphatase, β-glucuronidase, and glutamic-pyruvate transaminase. Immature male rats were castrated at 22 days of age and fed either a 20 percent casein diet or a protein-free diet for 13 days. After 10 days on the diets, half the rats in each group received a single subcutaneous injection of 0.25 mg of testosterone propionate. Increase in seminal vesicle weight was impaired by the lack of dietary protein. β-Glucuronidase and transaminase enzyme concentrations were not increased, and the response of the phosphatase enzyme was subnormal (tables 4 and 5). Increase in weight of the ventral prostate by 100 percent in response to

TABLE 4.—Response of β-glucuronidase of seminal vesicle to androgen in castrated rats

Diet (13 days)	Testosterone propionate (mg)	β-Glucuronidase	
		Units per g	Total
Protein-free	0	1,220	9. 5
	0. 25	1,598	36. 5
18% Casein	0	1,830	17. 0
	0. 25	2,673	77. 7

Table 5.—Effect of diet and androgen on accessory sex organ weight and alkaline phosphatase

Diet (13 days)	Testosterone propionate (mg)	Seminal vesicle weight (mg)	Alkaline phosphatase (μg/mg)	Ventral prostate weight (mg)	Alkaline phosphatase (μg/mg)
20% Casein	0	6. 7	5. 5	11. 3	3. 8
	0. 25	26. 8	16. 5	21. 9	12. 8
0% Casein	0	6. 0	4. 1	5. 0	4. 0
	0. 25	13. 6	7. 0	12. 3	8. 3

androgen was recorded in the presence and absence of dietary protein. However, in rats fed a protein-free diet, the tissue enzyme responses were subnormal or lacking.

Further studies have been conducted with rats fed a protein-free diet for 10 days followed by protein feeding for the last 3 days. Thus, protein was available to the rats from the diet at the time the administered steroid was acting. Adding ethionine to an 18 percent casein diet or substituting gelatin for casein reduced organ responses to androgen (table 6). These data provide further support for the treatise that nutrition can modify hormone effectiveness.

Table 6.—Effect of diet on accessory sex organ response to androgen in castrated rats

Diet	Testosterone propionate (mg)	Seminal vesicle weight (mg)	Ventral prostate weight (mg)
Initial control	0	11. 1	16. 9
Protein-free for 10 days	0	6. 2	5. 7
+ 18% Casein for 3 days	0. 25	16. 6	13. 5
+ 18% Casein + ethionine	0	5. 0	4. 3
	0. 25	10. 6	6. 9
+ 18% Gelatin	0	3. 6	3. 5
	0. 25	8. 8	7. 5

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DISCUSSION

Dr. Brandes: I would like to point out to Dr. Leathem that in rats on a restricted diet the changes in the prostate as seen in the electron microscope greatly resemble those in the castrated animal.

Dr. Mann: There is, I am glad to say, close agreement between the observations made by Dr. Leathem on the diminished response of nutritionally defective rats to androgens and our results based on experiments with castrated monozygous bull calves. On injecting the same quantity of testosterone to twin calves, one normally fed and the other kept on restricted food intake, we observed that the underfed calf secreted much less fructose and citric acid, in the electrically induced ejaculates, than the control twin. This was so in spite of the fact that, owing to underfeeding, the experimental animal had a lesser body weight and was therefore receiving more testosterone per kilogram of body weight. Apparently the diminished response of an underfed castrated animal to testosterone is in some way related to the action of the pituitary gland which, however, is not mediated by the gonads. Perhaps we should in our future experiments consider more seriously the possibility envisaged by Dr. Grayhack that accessory organs can respond to stimuli emanating directly from the hypophysis. We might do that by comparing the response to androgen of a hypophysectomized castrated animal with one in which the testes have been removed but the pituitary gland left intact. Have you perhaps started some experiments in that direction?

Dr. Leathem: I certainly agree with you and indeed have incorporated in the manuscript the fact that consideration must be given to the pituitary factors. Certainly in protein depletion we are reducing the level of growth hormone. We have done one experiment in hypophysectomized animals in which we initially protein-depleted the animals for a 40-day period, then removed the pituitary, and continued the animals thereafter by feeding them either protein or no protein. A highly significant difference between the two groups was obtained in response to a standard dose of androgen. One of the difficulties encountered is the degree of depletion, as we have been finding many purchased animals actually in a state of partial depletion when we buy them. I should like to go back and look at what growth hormone and lactogenic hormone do in these depleted animals, with and without pituitary glands. The other question is whether there is any adrenal contribution.

Dr. Segaloff: I would like to point out that some of your observed effects can be seen in dehydrated animals. I wonder if one of the things you are doing with your high level protein feeding is to cause tissue dehydration?

Dr. Leathem: I am afraid I cannot answer that.

Dr. Scott: I think most of you remember the experiments of Huggins and Pazos, to which Dr. Leathem has just referred, in which either chorionic gonadotropin or androgen administered to the starved Huggins' fistula dog would restore prostatic secretion but not salivary secretion.

Dr. Leathem: Dr. Scott, in Dr. Huggins' work, is it possible that if other chemical endpoints had been measured in the prostatic fluid there would not have been complete restoration?

Dr. Scott: I cannot answer that. I think that the volume of prostatic secretion is as accurate a measurement of androgen stimulation as is the measurement of total solids, total nitrogen, etc., in this fluid. To my knowledge only volume measurements were reported in the experiment on starvation by Huggins and Pazos.

Dr. Lasnitzki: Mrs. Pavich, who worked in our laboratory, found that the isolated rat pituitary in culture continued to function in a defined medium containing only 5 percent serum. The criterion of effect was the stimulation of follicle cells in the ovary. The ovary and the pituitary were grown in close contact, and there was a definite effect over the 7 days the experiment lasted.

Dr. Leathem: I was not aware of this. Thank you.

Dr. Mann: Dr. Segaloff mentioned the distinct possibility that loss of water by a male accessory tissue may be a decisive factor in determining the response of that tissue to androgenic stimulation. I should like to draw the attention to some other factors that might conceivably affect the androgenic response of an accessory organ in a noncastrated, but sexually immature, male. During experiments made in collaboration with my colleague L. E. Rowson in Cambridge, we observed that bull calves at the age of 3 months, whether normally fed or underfed, still lack the ability to secrete fructose or citric acid in electrically induced ejaculates. Bull calves normally begin to secrete large quantities of fructose and citric acid only at about 5 to 6 months, and spermatozoa appear in their ejaculates at about 8 to 9 months of age. On injecting a given dose of chorionic gonadotropin into 3-month-old twin calves, one of which was fed normally and the other underfed, we found that the secretion of fructose and citric acid was initiated in both animals, but to a different degree: There was much more fructose and citric acid in the electrically induced ejaculates from the normally fed than from the underfed twin. We next repeated the experiment using a younger pair of twins and found that when gonadotropin is injected too early, a calf, either normally fed or underfed, is incapable of secreting fructose and citric acid, in spite of the fact that at that stage of development testosterone can already be detected in the testes. These observations lead me to suspect that the responsiveness of the accessory organs in an intact but very young male may be restricted not so much by the amounts of testosterone actually available in the testes, as by the inadequate rate of testosterone release. It is also conceivable, though by no means demonstrable, that the enzyme systems responsible for the production of fructose or citric acid are underdeveloped in the accessory organs of immature males.

Dr. Kochakian: In building a tissue you need amino acids to make the protein, so it would be desirable to have the right amino acids to build the particular protein. We have not analyzed rat prostate but we have analyzed guinea pig prostate and seminal vesicles, and these tissues require all the amino acids in their growth under androgen stimulation. Therefore, if the amino acids are not available from dietary sources, the body can provide them from endogenous sources. I wonder what would have happened if you had given a larger dose of testosterone over a longer period. Would you get a reshuffling of the protein in the body to give a response in the prostate of the dietary deficient rat equivalent to that in the normal animal?

Dr. Leathem: Our studies were concerned with one dosage and one time period, and we do not know what protein shifts would be anticipated with other dosages. I think that whether these shifts can or cannot occur would be contingent upon the degree of undernutrition and the amino acid availability. In fact, I would like to return the question to Dr. Kochakian and ask how much we know about the metabolic pool of nitrogen in relationship to the seminal vesicle. We think about a free exchange of amino acids between liver and nitrogen pools, but how much do we know about the accessory organs and their willingness to give up nitrogen in depletion or to take it back in their own growth?

Dr. Kochakian: I published something on this about 15 years ago. When androgen is administered to the rat, there is stimulation of growth of the seminal vesicles, prostates, kidneys, and body weight, but with prolonged treatment a priority is set up in which the seminal vesicles and prostate continue to grow at the expense of other tissues, especially muscle, rather than by continued use of dietary proteins.

HORMONAL FACTORS IN THE ADRENAL-DEPENDENT GROWTH OF THE RAT VENTRAL PROSTATE[1]

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A relationship between the adrenal glands and changes in the male sex accessories of man has been recognized for many years (*1*). The adrenogenital syndrome is characterized by adrenal cortical hyperplasia or adrenal tumors that produce androgens.

However, experimental results with laboratory animals, particularly the rat, have been the subject of controversy since the report of Davidson and Moon (*2*) that the weights of the sex accessories in immature castrated male rats are increased by adrenotropic extracts. Nelson (*3*) reported similar effects of adrenocorticotropic hormone (ACTH) on the sex accessories of young male and female rats in the absence of the gonads and the pituitary gland. However, Moore (*4*) and, later, Vanderlaan (*5*) were unable to confirm the androgenic effects of ACTH.

Although ACTH is generally considered to be the anterior pituitary hormone primarily responsible for stimulation of adrenal androgen synthesis, there is reason to believe that other anterior pituitary hormones may also be involved in androgen synthesis or end organ responses observed. Evidence that growth hormone (STH) enhances the action of ACTH in maintaining the sex accessories of hypophysectomized-castrated male rats has been obtained by Lostroh and Li (*6*).

We have studied the gravimetric response and histological effects of exogenous ACTH alone and in combination with other anterior pituitary hormones, and thyroxine, on the ventral prostate gland of castrated, castrated-adrenalectomized, and castrated-hypophysectomized male rats. Evidence of a synergistic relationship between ACTH and prolactin is presented.

MATERIALS AND METHODS

Male Sprague-Dawley rats were used in all experiments. Number of animals and age at time of surgical procedures (bilateral orchiectomy, bilateral orchiectomy-adrenalectomy, bilateral orchiectomy-hypophysectomy) are shown in tables. Stock diet was fed *ad libitum*. In addition, the rations of hypophysectomized rats were supplemented with a meat, bread, and milk mixture. A 1-percent saline solution was given to the adrenalectomized animals. Highly purified porcine ACTH (Acthar Gel, Armour & Co., Chicago, Ill.) in 16 percent gelatin was used in all experiments except as noted in table 5. Prolactin (ovine lactogenic hormone, Endocrinology Study Section, National Institutes of Health, Bethesda, Md.), growth hormone (somatotropin, bovine growth hormone, Endocrinology Study Section, National Institutes of Health, Bethesda, Md.), and luteinizing hormone (LH, Wilhelmi, lot XI–28–2) were given in aqueous solution. DL-Thyroxine was administered as an alkaline aqueous solution. All injections were given subcutaneously in separate sites. Animals were killed by exposure to chloroform. Initial and terminal body weights were recorded. At autopsy, wet weights of ventral prostate, seminal vesicles, and adrenals were recorded. Seminal vesicle secretion was expressed by blotting before weighing. Animals were examined for completeness of the several operative procedures. Tissues were fixed in Bouin's solution and stained by routine hematoxylin and eosin.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

[3] I wish to thank Dr. Roy Hertz for his advice and Mr. Donald L. Barber for his invaluable assistance.

RESULTS

In a series of preliminary studies, large doses of ACTH were given to immature rats that had been castrated 3 days before the beginning of treatment. In the series shown in table 1, the treated group of rats received 20 USP units of ACTH daily for 14 days. In spite of the profound catabolic effects noted in the terminal body weights of the ACTH-treated rats, there was a significant adrenal weight increase accompanied by a ventral prostate response which, on an absolute basis, was more than double that of the untreated rats and showed a fourfold increase when considered in terms of mg per 100 g of body weight. The adrenal dependence of the prostate effects is illustrated by the lack of ACTH stimulation of ventral prostate growth in adrenalectomized-orchiectomized male rats, as seen in data presented in table 2.

Since immature rats were used in our previous experiments, the effects of large doses of ACTH were next investigated in several series of bilaterally orchiectomized adult rats. Table 3 shows results from one of these groups in which adult animals were castrated at 5½ months and started on ACTH treatment 3 months later. The data indicate a ventral prostate response to ACTH treatment similar to that observed in the orchiectomized immature rats.

We next considered the possible effects of other anterior pituitary hormones on the secondary sex accessories of the orchiectomized rat. In table 4 we have compared the action of bovine growth hormone (STH) and ovine prolactin when each was given alone and when in combination with ACTH. Although prolactin alone had no effect on the ventral

TABLE 1.—Ventral prostate response to ACTH in young castrated male rats*

Treatment	Daily dose	Weight:					
		Initial body (g)	Final body (g)	Adrenal (mg)	Seminal vesicle (mg)	Ventral prostate (mg)	Ventral prostate (mg/100 g/ body weight)
ACTH†	20 USP units	47 ± 4	58 ± 4	61 ± 6	8 ± 1	17 ± 6	30 ± 12
None	—	50 ± 5	108 ± 2	30 ± 3	7 ± 1	8 ± 2	7 ± 2

*Five rats per group, castrated at 21 days, treatment started at age 24 days. Weights: mean and standard deviation.

†Given in divided doses twice daily for 14 days.

TABLE 2.—Lack of ventral prostate response to ACTH in castrated-adrenalectomized male rats*

Treatment	Weight: Body (g)	Weight: Ventral prostate (mg)	Weight: Seminal vesicle (mg)
ACTH (20 USP units)†	122 ± 7	8 ± 2	8 ± 1
None	135 ± 20	8 ± 3	10 ± 1

*Four rats per group, age 25 days at castration, adrenalectomy at 39 days. Weights: mean and standard deviation.

†Given in divided doses twice daily for 14 days, beginning 2 days after adrenalectomy.

TABLE 3.—Ventral prostate response to ACTH in castrated adult male rats *

Treatment	Weight: Body (g)	Weight: Adrenal (mg)	Weight: Ventral prostate (mg)	Weight: Seminal vesicle (mg)
ACTH (20 USP units)†	349 ± 17	178 ± 20	82 ± 16	140 ± 9
None	351 ± 30	72 ± 9	36 ± 1	121 ± 14

*Four rats per group, castrated at 5½ months, autopsied at 260 days of age. Weights: mean and standard deviation.

†Given in divided doses twice daily for 14 days.

prostate, an augmentation of the ACTH-induced growth response was observed with the concomitant administration of prolactin. Seminal vesicle weights were not affected by either treatment. STH alone or in combination with ACTH did not alter ventral prostate or seminal vesicle

TABLE 4.—Effects of ACTH, STH, and prolactin on ventral prostate of castrated male rats*

Treatment†	Daily dose	Weight: Body (g)	Weight: Adrenal (mg)	Weight: Ventral prostate (mg)	Weight: Seminal vesicle (mg)
None	—	199 ± 8	49 ± 4	9 ± 1	13 ± 1
ACTH	20 USP units	140 ± 9	116 ± 12	14 ± 2	9 ± 1
ACTH +STH	20 USP units 1.0 mg	130 ± 12	121 ± 17	10 ± 1	13 ± 3
STH	1.0 mg	185 ± 23	53 ± 2	10 ± 2	13 ± 4
ACTH +Prolactin	20 USP units 1.0 mg	137 ± 11	120 ± 9	21 ± 5	13 ± 2
Prolactin	1.0 mg	200 ± 13	46 ± 8	9 ± 3	13 ± 1

*Five rats per group. Weights: mean and standard deviation. Castrated at 24 days, treatment started at 46 days.

†Given in divided doses subcutaneously, twice daily for 14 days.

weights. In a second series (table 5), the synergistic effect of ACTH and prolactin was emphasized by the use of an ACTH dose which did not affect ventral prostate weight. When prolactin was given together with this level of ACTH, a significant prostate response occurred. Luteinizing hormone (LH) was ineffective under the same conditions.

TABLE 5.—Prolactin augmentation of ventral prostate weight (ACTH subthreshold) in castrated rats*

Treatment	Daily dose	Weight: Final body (g)	Adrenal (mg)	Ventral prostate (mg)	Seminal vesicle (mg)
None	—	133 ± 10	34 ± 8	9. 0 ± 1. 8	9. 9 ± 0. 9
ACTH†	5 USP units	138 ± 6	45 ± 5	9. 3 ± 1. 8	10. 2 ± 1. 6
ACTH +Prolactin‡	5 USP units 1 mg	149 ± 24	49 ± 7	23. 5 ± 4. 8	13. 0 ± 4. 2
ACTH +LH‡	5 USP units 0.2 mg	141 ± 13	44 ± 6	7. 8 ± 1. 4	9. 4 ± 2. 7
LH	0.2 mg	150 ± 12	35 ± 3	9. 3 ± 1. 8	9. 2 ± 1. 9

*Five rats per group. Weights: mean and standard deviation.

†Given once daily for 14 days; ACTH in sesame oil (Acthar Gel, Armour & Co., Chicago, Ill., lyophilized).

‡Prolactin and LH given in divided doses, twice daily for 14 days.

Data presented in table 6 further confirm the effects noted when a threshold dose of ACTH was given. Although adrenal weight was decidedly increased by the parenteral administration of 8 USP units of ACTH, addition of prolactin was a prerequisite for the ventral prostate responses observed.

In the preceding experiments, the presence of the anterior pituitary gland presented problems in interpretation of hormonal factors contributing to the responses observed. In the next group of experiments, hypophysectomized-orchiectomized rats were used to determine whether the effects on prostate weight could be reproduced under conditions of limited hormonal replacement. Treatment was begun on the day following castration in a group of animals hypophysectomized when 23 days old and orchiectomized at 27 days. Results are presented in table 7. Replacement with ACTH alone, ACTH plus prolactin, and prolactin alone produced no marked weight alteration in ventral prostate or seminal vesicles, though prolactin appeared to augment the adrenal weight response to ACTH. In other experiments, higher doses of ACTH and prolactin continued to exhibit a comparable lack of stimulation. However, in the next series (table 8), when DL-thyroxine was combined with ACTH and prolactin, ventral prostate weight was twofold greater than that of untreated animals. ACTH plus thyroxine was ineffective. DL-Thyroxine was used in these experiments because highly purified thyrotropin was not available.

TABLE 6.—Prolactin augmentation of prostate growth in castrated male rats*

		Weight:				
Treatment†	Dose	Initial body (g)	Final body (g)	Adrenal (mg)	Ventral prostate (mg)	Seminal vesicle (mg)
None	—	128 ± 2	186 ± 6	50. 5 ± 7. 0	8. 7 ± 1. 0	12. 0 ± 3. 5
ACTH + Prolactin	8 USP units 2 mg	125 ± 4	152 ± 12	85. 2 ± 3. 5	16. 3 ± 3. 2	12. 4 ± 1. 6
ACTH	8 USP units	128 ± 4	154 ± 11	89. 7 ± 8. 6	8. 7 ± 1. 8	11. 0 ± 1. 4
Prolactin	2 mg	129 ± 2	172 ± 23	56. 0 ± 10. 0	10. 5 ± 1. 3	12. 3 ± 1. 5

*Five rats per group. Weights: mean and standard deviation.
†All injections given twice daily subcutaneously for 12 days.

Table 7.—Effects of ACTH and prolactin in hypophysectomized-castrated male rats*

Treatment	Daily dose†	Number of rats	Weight: Final body (g)	Weight: Adrenal (mg)	Weight: Ventral prostate (mg)	Weight: Seminal vesicle (mg)
ACTH	8 USP units	5	71 ± 9	28.1 ± 1.8	5.4 ± 1.3	7.7 ± 1.0
ACTH + Prolactin	8 USP units 1 mg	5	74 ± 4	41.4 ± 2.5	7.1 ± 0.7	7.9 ± 1.3
Prolactin	1 mg	4	88 ± 10	8.5 ± 1.4	6.3 ± 1.2	7.9 ± 1.0
None	—	5	76 ± 12	9.9 ± 0.7	4.5 ± 1.5	6.1 ± 0.8

*Weights: mean and standard deviation.

†Given in divided subcutaneous doses for 14 days.

Table 8.—Effect of thyroxine in hypophysectomized-castrated male rats following treatment with ACTH and prolactin*

Treatment †	Total daily dose	Weight: Final body (g)	Weight: Adrenal (mg)	Weight: Ventral prostate (mg)	Weight: Seminal vesicle (mg)
None	—	69 ± 7	14.2 ± 0.9	7.1 ± 0.8	8.0 ± 1.2
ACTH + Prolactin + DL-Thyroxine	16 USP units 1 mg 10 μg	58 ± 3	48.1 ± 6.5	14.3 ± 1.2	9.9 ± 0.7
ACTH + DL-Thyroxine	16 USP units 10 μg	52 ± 2	32.5 ± 1.9	9.0 ± 2.4	6.0 ± 0.8

*Five rats per group, hypophysectomized at 23 days, castrated at 28 days.

†Given in divided doses twice daily subcutaneously for 8 days.

The histological appearance of sections of ventral prostate from this group is shown in figures 1 and 2. The marked atrophy of acini and stroma (fig. 1) is in marked contrast to the distended acini lined with tall columnar epithelium and prostatic secretion within some of these acini (fig. 2) from the hypophysectomized-orchiectomized male rats receiving ACTH, prolactin, and DL-thyroxine.

Finally, when rats received 32 USP units of ACTH plus 1 mg of prolactin daily shortly after hypophysectomy and orchiectomy, the mean ventral prostate weight response was 4 times that of the untreated animals (table 9). ACTH used in these experiments contained less than one milliunit of thyrotropin in 80 USP units.[4]

DISCUSSION

These investigations have confirmed the positive findings of others with respect to the androgenic effects of adrenocorticotropin in castrated male rats (*2*, *3*). The lack of ventral prostate response to ACTH in the castrated male rat as reported by Moore (*4*) could be attributable to the low dose of ACTH. In the present experiments, a similar dose was also ineffective except when given in combination with prolactin. The findings of Vanderlaan (*5*) cannot be explained. However, the strain of rat used was not identified, and this is an important factor in the rat ventral prostate response to androgen as noted by Lostroh and Li (*7*).

Price (*8*) observed that when rats were castrated a few days after birth the ventral prostate continued to develop and did not begin to undergo castration atrophy until 4 to 5 weeks post partum. In further studies, Price (*9*) concluded that the ventral prostate of the young castrated male rat was stimulated by endogenous adrenal androgens.

A local adrenal androgenic action on the histological structure of the rat sex accessories was described by Price and Ingle (*10*). A weak androgenic effect was noted after adrenal autotransplantation in seminal vesicles or ventral prostates of castrated males.

From the results obtained after combined treatment with ACTH and other anterior pituitary hormones, it is evident that prolactin complements the ventral prostate weight response to exogenous ACTH in the orchiectomized male rat. The synergistic effect of prolactin was most clearly observed when threshold levels of ACTH were given.

Further experiments, in which hypophysectomized-orchiectomized male rats were used, indicated that thyroxine was necessary for the ventral prostate effects of ACTH and prolactin in the absence of the anterior pituitary. In subsequent experiments it was noted that ACTH plus prolactin effectively prevented the prostatic atrophy resulting from hypophysectomy and orchiectomy when the dose of ACTH used in the preceding study was doubled. It is unlikely that the thyrotropin activity

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[4] Bates Chick Assay, through the kindness of Dr. William Odell.

Table 9.—Effects of ACTH and prolactin in hypophysectomized-castrated male rats*

Treatment	Daily dose	Weight:				
		Initial body (g)	Final body (g)	Adrenal (mg)	Ventral prostate (mg)	Seminal vesicle (mg)
ACTH	32 USP units	46 ± 1	47 ± 4	39.2 ± 5.3	8.3 ± 1.7	6.1 ± 1.7
ACTH + Prolactin	32 USP units 1 mg	46 ± 2	51 ± 2	51.4 ± 10.8	16.5 ± 1.6	5.3 ± 0.6
No treatment	—	46 ± 2	65 ± 2	8.3 ± 0.3	4.1 ± 0.2	5.5 ± 0.1

*Five rats per group, hypophysectomized at 23 days, castrated at 25 days. Treatment started on day 25 (10 days' treatment). Weight: mean and standard deviation. TSH content of ACTH—less than 1 milliunit per 80 units ACTH (Bates Chick Assay).

present in this dose of ACTH would stimulate sufficient thyroid hormone secretion to account for the ventral prostate weight increase observed. The shortened interval between hypophysectomy and the initiation of treatment might account for these effects, since the adrenal cortex is less sensitive to ACTH as the postoperative interval increases.

Throughout these experiments, it was obvious that the seminal vesicles were relatively unresponsive when compared with the ventral prostate. The greater sensitivity of the rat ventral prostate to androgenic secretory products of the adrenal cortex was described by Price and Ingle (*10*).

In conclusion, we have confirmed earlier findings that the rat ventral prostate response to large doses of ACTH is adrenal-dependent. Furthermore, we have demonstrated that prolactin augments the effect of ACTH with respect to weight and secretory activity of the prostate. This was seen both in the orchiectomized and the hypophysectomized-orchiectomized male rat. However, these observations apply only to a hyperactive adrenal gland. Whether the synergistic action of prolactin is mediated through the adrenals or exerted directly on the prostatic tissue, such as the augmenting effects of prolactin on the rat prostate response to androgen (*11*, *12*), is a question to be resolved.

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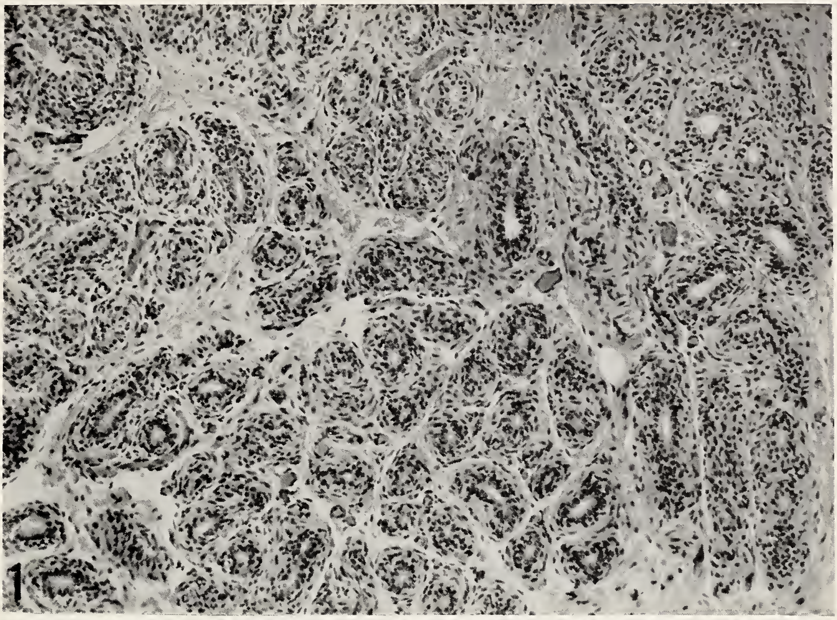
PLATE

Plate 31

Figure 1.—Section of ventral prostate from untreated hypophysectomized-castrated male rate. *Note* atrophic acini. × 130

Figure 2.—Section of ventral prostate from hypophysectomized-castrated male rat treated with ACTH, prolactin, and DL-thyroxine. *Note* acinar development including columnar epithelium and prostatic secretion. × 130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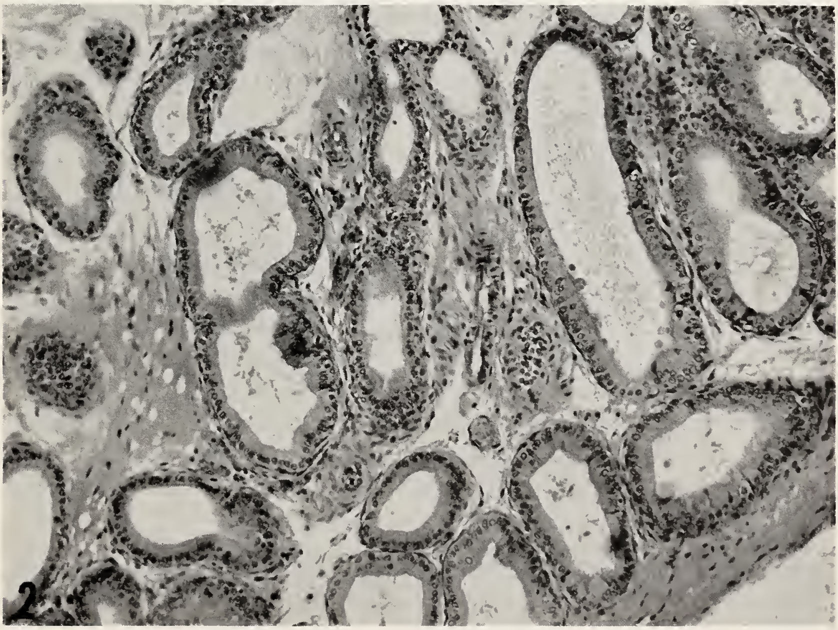
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DISCUSSION OF PAPERS ON GROWTH AND DEVELOPMENT; EFFECT OF HORMONES ON CARBONIC ANHYDRASE [1]

GREGORY PINCUS, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

First it is obvious that the section concerned with growth and function has posed several problems, which I would like to review briefly. Dr. Scott made an extremely valuable contribution in describing the nature of the human prostate and, particularly, the differentiation of function as it varies with prostate morphology. I was especially impressed by the fact that benign prostatic hypertrophy and cancer of the prostate occur in morphologically dissimilar tissues. In a way, we have here the central problem of this Conference.

The prevailing concept has been that since the prostate is an androgen-sensitive organ *par excellence*, any derangement in its function may reflect either derangement of androgen function or its responsivity to androgens. This is where Dr. Gallagher's contribution is invaluable. He pointed out that this concept has in part been based upon steroid excretion data, the imperfections of which were made obvious by his very careful analytic study. His interpretation attributes importance to what we might call the alarm reaction in the human which alters these excretion ratios and the total excretion of steroids. However, a critical test of the significance of this has not been made, as I believe Dr. Gallagher recognizes. One of the discussants stated that the administration of ACTH to these patients might give us a little more information than the direct analysis of the steroids. Dr. Dorfman indicated that the true nature of androgen production in these patients still remains to be established.

The other problem which was stressed, particularly by Dr. Saunders and to some extent by Dr. Dorfman, is what steroids really stimulate the prostate, and here the function-structure relationship is most important. It has been made clear that you may stimulate the prostate in experimental animals by a variety of steroids. It has been claimed that perhaps steroids which are most important in abnormal prostatic growth and abnormal prostatic function have not been identified. They may in fact be represented by structural metabolites not yet perfectly established, and certainly Dr. Saunders' data give much food for thought in this direction.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

Is it possible that there are indeed such metabolites circulating in the blood of individuals with abnormal prostatic functions? Dr. Dorfman's contribution also indirectly points to this possibility. He has shown that the inhibition of the characteristic androgen effect is not necessarily accomplished by conventional steroids. It is a commonplace that the estrogens will act as antiandrogens, and that therefore these may oppose what may be going on in a number of "hyperandrogenic" states. It is also obvious that there are neutral steroids that oppose androgen action. Some of these neutral steroids bear a strong resemblance to naturally occurring steroids and some of them (for example, certain progestins) behave like well-known, naturally occurring steroids.

I would like to interject one remark: There may be much more production of progestin in the human organism than we have recognized. For example, in some recent studies conducted in our laboratories on the secretion rates of progesterone, the rates in the human male are very respectable; they vary from 3 to 6 mg per day, and this is indeed a high production of active progesterone. In any event, the possible role of progestins as antagonists to androgens still remains to be established.

It should be noted that the papers on the pituitary, those of Dr. Li and Dr. Grayhack, again indicate there may be more than a simple androgen-prostate interrelationship. For example, referring to Dr. Tullner's contribution, we have the possibility of steroids originating from the ACTH target organ, the adrenal, which may intervene in the response of the prostate. Second, normal responsivity unquestionably requires normal thyroid function. Finally, we have strong evidence that growth hormone and prolactin may have a direct effect on prostatic tissues. Although some of this has been pointed out previously, the confirmation and repetition in a critical way of these early observations, I think, deserve congratulation.

Dr. Leathem has brought up several problems that the endocrinologist ordinarily eschews because he likes to think his rats or mice are well nourished and therefore depends, perhaps a little too much, on so-called standard laboratory conditions. What is the contribution of nutrition to the whole response of this very sensitive target organ for the steroid hormones, and, as we have seen, to some of the pituitary hormones? Until we can get to the basic phenomena involved (what is the nature of this growth, of this response of these tissues to the stimulating hormones) we will have a very fine collection of data, the ultimate explanation for which still must be found. In this connection, I shall present our data pointing up what has been obvious from Dr. Leathem's data and throughout this meeting: There are aspects of growth and function which had better be scrutinized very carefully before we think that one or the other is entirely independent.

Table 1 shows data published by Dr. Miyake on the effects of castration and testosterone on the carbonic anhydrase activity of the seminal vesicles and of the ventral prostate of immature rats. What rather surprised us was that, although there was an excellent weight response to testosterone in both the seminal vesicles and the prostate compared to the controls, the change in the enzyme activity was quite different in the two organs. In the seminal vesicles, castration had no influence on enzyme activity and testosterone injection produced a marked increase in activity; in the ventral prostate, castration resulted in a significant increase in enzyme activity and testosterone treatment resulted in a decrease to that found in normal animals. Yet both glands responded in terms of growth practically identically to testosterone. This has us a little puzzled and we have since followed this more carefully.

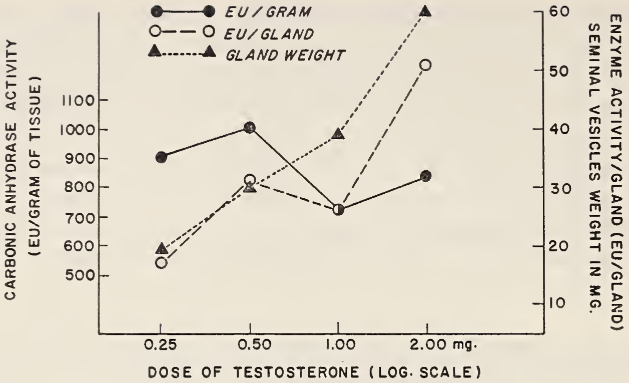
TABLE 1.—Effect of castration and testosterone on the carbonic anhydrase activity of the seminal vesicles and ventral prostates of immature rats (Miyake, T., and Pincus, G.: *Endocrinology* 65: 64–72, 1959)

Treatment	Number of rats	Average body weight (g)	Seminal vesicles		Ventral prostate	
			Average weight (mg)	EU/g wet tissue	Average weight (mg)	EU/g wet tissue
Normal (sesame oil)	4	113	21. 8	254	62. 0	34
	4	121	28. 4	295	72. 8	87
	4	90	16. 2	334	37. 3	44
	4	106	23. 9	313	59. 6	41
	4	105	19. 5	340	52. 1	44
	Mean	107	22. 0	307 ± 15. 5*	56. 8	50 ± 9. 2*
Castration + sesame oil	4	91	7. 0	357	14. 0	227
	4	86	7. 2	379	11. 3	111
	4	86	7. 5	291	9. 0	208
	4	91	7. 2	247	10. 0	238
	3	84	6. 3	280	8. 8	286
	Mean	88	7. 0	311 ± 24.7*	10. 6	214 ± 28. 8*
Castration + testosterone 2. 4 mg	4	95	93	676	109	32
	4	84	100	649	109	124
	4	85	96	490	98	62
	4	93	100	610	114	52
	4	87	106	562	122	105
	Mean	89	99	597 ± 33.0*	110. 4	75 ± 17. 1*

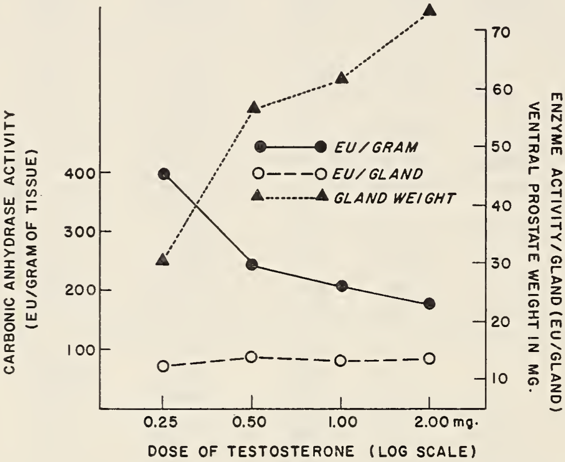
*Mean ± SE.

In text-figure 1 are data on the effect of varying doses of testosterone on the weight of the seminal vesicle, the enzyme content per gram, and the total enzyme activity in the gland. When you take into account the weight increase, you can see that the concentration here hardly changes, or, if anything, decreases, while the weight goes up quite nicely. The total amount of enzyme per gland does increase.

If you examine the same data for the ventral prostate (text-fig. 2), you will find a good weight increase, indicating excellent responsiveness to androgen. On the other hand, enzyme concentration in units per gram actually decreases, as was shown previously, while the total amount of enzyme per gland does not change at all. This suggests that there is good growth of the gland without a concurrent increase in enzyme activity.



TEXT-FIGURE 1.—Effect of testosterone on the carbonic anhydrase activity of seminal vesicles from castrated immature rats.

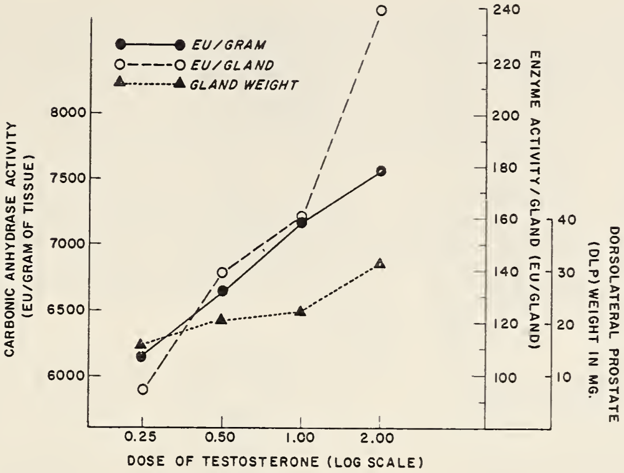


TEXT-FIGURE 2.—Effect of testosterone on the carbonic anhydrase activity of ventral prostrates from castrated immature rats.

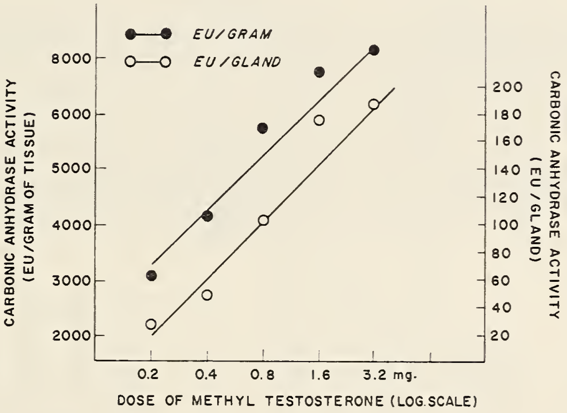
Text-figure 3 gives the data on the dorsolateral prostate which appears to be the only accessory organ where there is some consistency in the response. Increases in gland weight following testosterone are accompanied by an increase in enzyme concentration and in the total amount of enzyme that one finds in the gland. This response of the dorsolateral prostate was investigated more intensively.

Text-figure 4 presents data for methyltestosterone-treated rats, and a fairly good dose-response relationship, either in concentration of enzyme units per gram or in the total amount of enzyme per gland, is evident.

Table 2 shows the effect of progesterone on the enzyme activity of testosterone-stimulated dorsolateral prostates. The ventral prostate/body weight ratios in tables 2 and 3 were obtained from Dr. Dorfman's



TEXT-FIGURE 3.—Effect of testosterone on the carbonic anhydrase activity of the dorsolateral prostates from castrated immature rats.



TEXT-FIGURE 4.—Effect of methyltestosterone on the carbonic anhydrase activity of the dorsolateral prostates from immature castrated rats.

data. Progesterone produced only slight decreases in enzyme concentration and a significant decrease in enzyme activity per gland in only one case. The effect on the dorsolateral prostate weight was not consistent. Two other progesterone derivatives (table 3) produced clear depressions in the weights of both prostates. Enzyme concentrations were significantly reduced by the higher doses of both compounds, and enzyme activity per gland was reduced in all cases.

Table 2.—Effect of testosterone and progesterone on the carbonic anhydrase activity of the dorsolateral prostates from immature castrated rats

Treatment	Dorsolateral prostate weight (mg)	Ventral prostate/body weight	Carbonic anhydrase (units/g)	Carbonic anhydrase/gland	Number of determinations
S.S.V.,* control	4. 2	0. 14 ± 0. 015†	6, 036 ± 420†	25. 4	8
Testosterone, 2.0 mg	34. 23	1. 2 ± 0. 078	9, 374 ± 393	318. 3 ± 15. 6†	6
Testosterone, 2.0 mg + progesterone, 50 mg	25. 5	1. 01 ± 0. 108	8, 724 ± 838	230. 0 ± 47. 3	6
Testosterone, 2.0 mg + progesterone, 100 mg	23. 7	0. 94 ± 0. 030‡	9, 269 ± 431	219. 9 ± 22. 2	7
Testosterone, 2.0 mg + progesterone, 200 mg	30. 0	0. 86 ± 0. 061	8, 809 ± 267	265. 7 ± 25. 7	7

*Steroid suspending vehicle which consists of an aqueous solution of sodium chloride (0.9%), polysorbate 80 (0.4%), carboxymethylcellulose (0.5%), and benzyl alcohol (0.9%). All compounds suspended in S.S.V. and 0.5 ml per day given for 7 days.

†Mean ± SE.

‡Underlined values significantly different from testosterone, 2 mg.

Table 3.—Effect of testosterone and progestins on the carbonic anhydrase activity of the dorsolateral prostates from immature castrated rats

Treatment	Dorsolateral prostate weight (mg)	Ventral prostate/body weight	Carbonic anhydrase (units/g)	Carbonic anhydrase/gland	Number of determinations
S.S.V.,* control	4. 2	0. 14 ± 0. 018†	5, 236 ± 940	21. 8	8
Testosterone, 2 mg	21. 7	1. 07 ± 0. 096	11, 828 ± 480	253. 9 ± 14. 5†	10
Testosterone, 2 mg + 11α-hydroxyprogesterone, 150 mg	14. 32	0. 72 ± 0. 049‡	10, 886 ± 609	154. 7 ± 23. 5	8
Testosterone, 2 mg + 11α-hydroxyprogesterone, 300 mg	15. 0	0. 74 ± 0. 056	7, 304 ± 288	111. 8 ± 12. 9	10
Testosterone, 2 mg + 16,17-epoxyprogesterone, 100 mg	15. 8	0. 83 ± 0. 045	9, 635 ± 738	152. 3 ± 15. 6	9
Testosterone, 2 mg + 16,17-epoxyprogesterone, 200 mg	15. 8	0. 83 ± 0. 050	8, 770 ± 473	138. 7 ± 15. 6	10

*Steroid suspending vehicle which consists of an aqueous solution of sodium chloride (0.9%), polysorbate 80 (0.4%), carboxymethylcellulose (0.5%), and benzyl alcohol (0.9%). All compounds suspended in S.S.V. and 0.5 ml per day given for 7 days.

†Mean ± SE.

‡Underlined values significantly different from testosterone, 2 mg.

Table 4 deals with the effect of cadmium chloride, a known inhibitor of testis function in the rat. As was anticipated, the testes, ventral prostate and dorsolateral prostate weights were decreased as was the carbonic anhydrase activity of the dorsolateral prostate. Testosterone injections counteracted the effect of cadmium chloride.

TABLE 4.—Effect of $CdCl_2$ and testosterone on the carbonic anhydrase activity of the dorsolateral prostate of the immature, 21-day-old rat and on the weight of the ventral prostate and testes

Treatment	Testes weight (g)	Ventral prostate weight (mg)	Dorsolateral prostate weight (mg)	Dorsolateral prostate carbonic anhydrase activity
Control	385. 5	31. 0	9. 24	4365 ± 279. 2 (10)
$CdCl_2$ injection*	153. 7	13. 6	6. 92	2882 ± 278. 4 (10)
$CdCl_2$ + testosterone†	145. 4	39. 5	10. 72	5082 ± 134. 8 (6)
Testosterone	315. 4	42. 4	12. 98	6074 ± 247. 9 (10)

*$CdCl_2$ (0.03 M/kg) was injected on day 1 only.
†Testosterone was injected for 3 days (2 mg/0.3 ml oil), and the animals were killed on day 4.

In conclusion, I am sure we shall hear more about the problems of function versus growth structure as the program progresses. These may indeed be inseparable. They have many implications that require further explanation, and I think the major lesson I have learned from the papers in this section is that many phenomena relating to prostate function depend on the morphology of the tissues, on the potential response, and on the nature of the hormones under study.

BIOCHEMICAL CONSIDERATIONS

Chairmen: John C. Babcock and Albert Segaloff

BIOCHEMISTRY OF THE PROSTATE GLAND AND ITS SECRETION[1]

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The secretory function of the prostate gland and other male accessory organs of reproduction, and the chemical composition of the secretory fluids produced by these glands, have in the past been the subject of several reviews in which detailed information and references should be explored (*1–5*). An up-to-date account, listing additional references and discussing some of the more recent developments in the field of biochemistry of the semen and male reproductive tract, will be published shortly in the form of a monograph (*6*). Here, I wish to present a brief resumé of the salient facts concerning the biochemistry of the prostate gland, without cataloguing all the numerous papers which have been written on that subject, but, listing instead, the main biochemical features that distinguish the prostate and certain related glands from other organs of the animal.

The biochemistry of the prostate gland, though still in a fragmentary state, has received much attention during the last 3 decades, especially in relation to man, dog, and rat. In man it is a large organ, susceptible to certain pathological changes such as inflammation, benign hypertrophy, and cancerous metaplasia. On gross inspection the human prostate appears fairly uniform, but functionally its various regions are quite diverse. More detailed observation indicates that there are characteristic differences in the distribution of lipides between the ventral and dorsal region. Following the administration of diethylstilbestrol, the cells of the ventral prostate regress and lose their eosin-staining material more quickly than the corresponding cells in the posterior region. This obvious duality in function, in the response of lipides to diethylstilbestrol, may be related, as pointed out by Huggins, to the predisposition of the dorsal region of the human prostate to carcinomatous changes and the resemblance between the diethylstilbestrol-induced regression in the anterior region of the normal human prostate and the changes induced in the cancer of that region by "antiandrogens."

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LIPIDES

The lipides produced by the prostate gland of man are of special interest since they are not confined to the gland itself but appear also in the prostatic secretion. The average content of total lipide in the human prostatic secretion was shown by Scott to be about 0.3 g per 100 ml, made up of phospholipides and cholesterol. Only one part of this material, however, represents soluble lipide. The remainder occurs in the form of various globules, droplets or granules, commonly referred to as "lipide bodies," much studied by the early investigators of the human prostate, and in the German literature of the nineteenth century often referred to as "Lecithinkörnchen." However, these "lecithin granules," commonly regarded as responsible for the opalescence of the prostatic fluid, do not appear to consist of lecithin alone. According to some authors they may be related to certain larger bodies, known as "colostrum corpuscles" and "corpora amylacea." The colostrum corpuscles are probably macrophages packed with masses of lipide granules, staining strongly red with eosin. The corpora amylacea are small, soft, laminated spheroidal bodies, usually located in the larger ducts and acini of the prostate, and perhaps containing some cholesterol and split products of nucleoproteins. Although typical of the human prostate, corpora amylacea are also found elsewhere, particularly among the insectivores where their production is considered to be one of the main secretory functions of the prostate gland; they are not found either in the dog or the rat.

In the dog Huggins found only 30 to 40 mg of total lipide per 100 ml of the prostatic secretion. From more recent histochemical studies of the dog prostate, it would appear that lipides constitute a major component of the acinar epithelium and consist partly of phospholipides and cholesterol, and partly of neutral fat. Lipide-laden cells form also a highly characteristic component of secretory epithelia in male accessory organs of other animals. In the bull, a species on which our efforts have been concentrated, the tubuli of the seminal glands (seminal vesicles) abound in eosinophilic granular secretion. Phospholipides, of various kinds, are also present in the male accessory secretions and seminal plasma of other large farm animals and include some aldehydogenic lipide, known as plasmalogen. With Dr. Hartree I showed, however, that most plasmalogen (phosphatidal choline) occurs in the spermatozoa and not in the seminal plasma. Spermatozoa are also capable of splitting plasmalogen; they metabolize the split products, probably a mixture of fatty acids, as part-substrate of their endogenous intracellular respiration. Lysoplasmalogen, which is the fragment of plasmalogen remaining after fatty acid liberation, and containing only a fatty aldehyde bound by the unsaturated ether-linkage to glycerylphosphorylcholine, possesses, like lysolecithin, certain sperm-damaging properties; it lowers sperm motility, fructolysis, and respiration. It is also capable, like lysolecithin, of plasmolyzing the red blood cells.

Even a short account of lipides produced in male accessory organs warrants a reference to an interesting group of unusual fatty acids known as the prostaglandins. The elucidation of the chemical structure of these compounds, endowed with oxytocic potency and pharmacodynamic activity toward smooth-muscle organs and blood vessels, is largely due to Bergström and his colleagues in Sweden. The two main representatives of this group of compounds are "prostaglandin *E*" and "prostaglandin *F*." The former, $C_{20}H_{34}O_5$, probably has the structure of 2-(6-carboxyhexyl)-3-(3-hydroxyocten-1-yl)-4-hydroxy-cyclopentanone, and the latter, $C_{20}H_{36}O_5$, is a reduction product of the former. There is some indication that, unlike in the seminal plasma, at the site of their formation in the accessory organs, the prostaglandins do not occur as free fatty acids, but are present in bound form, as phospholipides, from which they can be released enzymatically by the action of a phospholipase *A*-like enzyme.

As might be expected the concentration and distribution of lipides in the prostatic secretion of man and animals are subject to considerable individual variations, possibly as results of different rates of biosynthesis and metabolic turnover in the gland itself. Like other metabolic processes in male accessory organs, those involving lipides are closely dependent on androgenic stimuli. For example, the respiration-coupled synthesis of long-chain fatty acids from acetate is lower in prostatic tissue from castrated rats than from normal, but can be restored by treating the castrates with testosterone. There is also some evidence that in minced tissue preparations from the prostate of castrated rats, the synthesis of both fatty acids and proteins can be enhanced under certain circumstances by the addition of quantities of testosterone of the order of 10^{-7}M.

Appreciable differences also exist between healthy human subjects and those with benign hypertrophy of the prostate or chronic prostatitis in the electrophoretic pattern of lipides and lipoproteins in the prostatic secretion. In the normal secretion apparently there are at least three electrophoretically discernible components, of which two are moving at about the same rate as β- and γ-lipoproteins, and the third does not move in the electric field and probably consists of "lipide bodies." In prostatic hypertrophy and chronic prostatitis the lipide content of the prostatic secretion is considerably increased, mostly because of the higher content of the electrophoretically inert lipide component.

PROTEINS, ENZYMES, AND AMINO ACIDS

In addition to lipoproteins, there are in the prostate gland and its secretion other conjugated proteins, including certain flavoproteins, mucoproteins, and metalloproteins. However, before we consider the nature and function of these proteins in detail, some general remarks about them must be made. In some species, *e.g.*, rabbit, secretory processes in the male accessory organs are accompanied by marked changes in the structure of the secretory epithelium, varying from desquamation to cell rupture with subsequent liberation of the cellular contents. This type of secretion is naturally very rich in material of intracellular origin. Probably this explains the remarkably high content in seminal plasma of flavoproteins and various oxidizing enzymes normally found within cells and tissues. To illustrate this, one may point to the presence of transaminases, lactic, malic, and isocitric dehydrogenase, and the intermediary enzymes of glycolysis, *e.g.*, phosphohexoseisomerase and aldolase (zymohexase) in the seminal plasma of man and certain animals.

When one considers the nature of seminal proteins of prostatic origin, or of proteins derived from other accessory organs of man, it is important to remember that in the human seminal plasma the content of protein soon after ejaculation undergoes rapid changes of proteolytic nature, as manifested by a progressive decrease in the concentration of nondialyzable protein nitrogen and a simultaneous accumulation of non-protein nitrogen, free amino acids, and at a late stage, free ammonia. Analyses of non-protein nitrogen in human semen show increases from the initial level of 30 mg per 100 ml, immediately after ejaculation, to 300 mg per 100 ml after 30 minutes' incubation at 37°. Electrophoretic studies on the proteins of human seminal plasma indicate that the content of these proteins diminishes spontaneously after ejaculation, due in the first instance to fragmentation into so-called proteoses, and at later stages to a virtually complete breakdown into amino acids, brought about by enzymes derived chiefly from the prostatic secretion. Therefore it is by no means easy to define a "normal" content and distribution pattern of proteins in human seminal plasma. Moreover, pathological conditions in the male accessory glands, especially in the prostate, can produce definite changes in that pattern and possibly influence also the rate of proteolysis.

Prostatic proteolytic enzymes are an important factor in the process of spontaneous coagulation and liquefaction occurring in human semen after ejaculation. That process is believed to involve three distinct stages: 1) the formation of a fibrin-like clot, by the action of a clotting enzyme produced by the prostate on a fibrinogen-like protein substance, contributed by the seminal vesicles, 2) fibrinolysis, *i.e.*, splitting of the fibrin by a plasmin-like enzyme, also of prostatic origin, but formed from an inactive precursor, and 3) subsequent proteolysis of the lysed fibrin, leading to the formation of free amino acids. Some of these proteolytic agents occur also in male accessory organs of certain animals; clotting enzymes in particular are widely distributed among animals, especially rodents. In the guinea pig, for example, the coagulation mechanism is believed to involve two enzymic reactions: 1) the action of the enzyme vesiculase secreted in the coagulating gland, on the protein procoagulase contributed by the seminal vesicles, with the resulting formation of the clotting enzyme proper or coagulase, and 2) the clotting of coagulinogen, a coagulable protein produced in the seminal vesicles, by the coagulase.

$$\text{Procoagulase} \xrightarrow{\text{Vesiculase}} \text{Coagulase}$$

$$\text{Coagulinogen} \xrightarrow{\text{Coagulase}} \text{Coagulated protein}$$

Studies on the enzymic reactions responsible for the coagulation of semen in the guinea pig and rat suggest that clotting depends on enzymic denaturation of protein and bears little resemblance to the clotting of blood fibrinogen by thrombin. The latter will not coagulate the vesicular protein, nor will coagulase coagulate blood fibrinogen. An anatomical separation between the coagulating enzyme and coagulable protein, similar to that established in guinea pig and rat, exists also in other animals, but species differ as to the localization of the enzyme and substrate. In certain monkeys coagulation is brought about by the secretion of the cranial lobe of the prostate acting on the secretion of the seminal vesicle. In some *Insectivora*, such as *Condylura* and *Parascalops*, the coagulating agent is believed to be secreted by the Cowper's gland, but the coagulable substrate by the prostate gland.

Fibrinolysin, the plasmin-like enzyme system present in the human prostate and seminal plasma, is thought to be the agent responsible for initiating the liquefaction of human semen, which usually takes place within about 20 minutes after coagulation. Apart from the enzyme proper, the system contains several other components. One of these is the "kinase," which Lundquist and his colleagues separated and purified. Another one is the "plasmin-activator," which differs, however, from the analogous substance in blood plasma because it is much more resistant to treatment with heat.

The process of proteolysis, supposedly initiated by fibrinolysin, is carried on by at least two other proteolytic agents, fibrinogenase and aminopeptidase. Fibrinogenase, which is more active in the canine secretion than in human secretion, bears some resemblance to chymotrypsin by being active toward casein and hemoglobin, as well as human and bovine blood plasma fibrinogen; it differs, however, from chymotrypsin in certain other respects and is not inhibited by diisopropylphosphorofluoridate. Aminopeptidase, highly active in the human seminal plasma, is capable of hydrolyzing peptides with the formation of free amino acids, of which the seminal plasma always carries a characteristically large amount. In addition to the aminopeptidase, human semen contains pepsinogen (the precursor of pepsin) and an arginine-ester hydrolyzing enzyme. The latter is also present in the canine prostatic secretion and in male accessory organs of rodents.

Seminal plasma and prostatic secretion are also renowned for elevated phosphatase activity. The principal phosphatase of the human prostate gland is the so-called acid phosphatase, now available in a state of high chemical purity. It is, however, still doubtful whether the acid phosphatase activity of the human prostate is due to a single protein or to several. Electrophoretic studies on aqueous extracts of the gland indicate the latter. Much work has been done on the relation between the level of acid phosphatase in the prostate and androgenic activity, and the available evidence is in agreement with the concept that the prostatic phosphatase is a significant secondary sex characteristic, under hormonal control of the testis. The enzyme is present in the normal as well as the hypertrophied gland, and occurs, at a lower level, in prostatic cancer. As in the normal prostate, certain forms of prostatic cancer respond characteristically to androgenic stimuli, and cancerous growth of that type can be suppressed by antiandrogenic therapy, such as castration or treatment with estrogens.

There is a whole range of phosphorus-containing compounds that can act as substrates for acid phosphatase. Some of these substances are hydrolyzed to yield inorganic phosphate. Others are involved in transferase reactions such as the transfer of a phosphate group from β-phosphoglycerol or phosphocreatine to glucose, which leads to the formation of 6-phosphoglucose, or the transfer of phosphate from amidophosphate to glycerol. Some phosphorus compounds, however, act as inhibitors of phosphatase, *e.g.*, polyestradiol phosphate. The enzyme is also inhibited by polymeric phosphates of phloretin, phlorrhizin, and hesperidin. Among interesting inhibitors of prostatic phosphatase is L(+)tartrate since this can be used to distinguish between phosphatases of prostatic and nonprostatic origin.

Two other distinct groups of seminal proteins are the metalloproteins and mucoproteins. A metal characteristic of the prostate is zinc, which was first noted by Gabriel Bertrand in the human prostate some 40 years ago. Zinc has been studied extensively in recent years, particularly by Mawson in Canada and Gunn in the United States. The mean zinc content of the human prostate gland is about 70 mg per 100 g dry weight, and that of the rat dorsolateral prostate is similarly high. Much more research is needed to establish satisfactorily the form and function of prostatic zinc. Some years ago, in collaboration with Keilin, I found that zinc occurs in blood corpuscles mainly as the zinc-protein carbonic anhydrase. High carbonic anhydrase activity is also characteristic of the rat dorsolateral prostate, but there it was shown by Mawson to account for not more than one tenth of the total zinc content. Little is also known as yet about the mechanism underlying the ability of the prostatic tissue either to bind or to release the zinc. It has been demonstrated convincingly that an injection of a radioactive inorganic zinc salt to male rats results in a rapid uptake of Zn^{65} by the lateral tips of the rat dorsolateral prostate, and that under *in vitro* conditions, Zn^{65} is promptly taken up and concentrated by slices of the rat prostate. Castration of the rat is followed by a decrease, and testosterone administration by an increase, in the rate of Zn^{65}-uptake by the intact gland.

Another metal that occurs in the prostatic secretion in a protein complex is copper. The dog prostatic secretion is particularly rich in that metal. A partially purified copper protein has been obtained by us from the ram seminal plasma, but its relation to other copper proteins of the animal body, *e.g.*, hemocuprein or ceruloplasmin, still remains to be established.

As regards seminal mucoproteins, that is, proteins conjugated with mucopolysaccharides, so far only a few have been properly purified, chemically characterized, and examined with respect to their origin.

The available data make it clear, however, that the seminal plasma of man and animal contains more than one mucinous component. It is interesting that, as a rule, men excrete in urine significantly more mucoprotein than women. This sex difference must be due to an admixture of accessory secretions in male urine, but it is not known to what extent this mucoid material originates in the prostate gland. One distinct type of seminal mucin, in man as well as in animals, is associated with the presence of sialic acid. This type of mucoprotein has been investigated most extensively in the boar, where it was shown by E. F. Hartree to be secreted chiefly by the bulbourethral glands. Human seman contains about 100 mg of protein-bound sialic acid, largely derived from the seminal vesicle secretion and only to a small extent from the prostate. Chromatographically, seminal sialic acid behaves like *N*-acetylneuraminic acid; it can be released from the protein complex by treatment with the enzyme neuraminidase.

An important but as yet unsolved problem concerns the mechanism by means of which proteins and enzymes are synthesized in the prostate, presumably under androgenic stimulation. So far, there is a lack of experimental support for the concept that testosterone exerts its activating influence selectively, *i.e.*, by facilitating the formation of a particular enzyme, or single protein. It is more probable that the synthesis of proteins in general, rather than that of any individual component, is promoted by testosterone. If so, then the protein synthesis in the prostate gland may be intimately linked with the synthesis of nucleic acids, and the explanation for the generally high rate of synthetic reactions should perhaps be sought in reactions involving nucleic acid.

NUCLEIC ACIDS AND NUCLEOLYTIC ENZYMES

Dr. C. D. Kochakian and Dr. H. G. Williams-Ashman will discuss later the various aspects of the biosynthesis of nucleic acid and the nucleic acid-protein relationship in prostatic tissue. But I would like to add a brief reference to yet another facet of nucleic acid biochemistry: the occurrence in male accessory secretions of metabolites of nucleic acids, and certain enzymes which break down nucleic acid, nucleotides, and nucleosides.

It is typical for the secretions of the male accessory organs to exhibit not only nuclease but also nucleotide pyrophosphatase activity. In addition, they contain a number of coenzyme-splitting enzymes including several adenosinetriphosphatases (one of which forms inorganic pyrophosphate), the so-called 5-nucleotidase (which dephosphorylates adenine-5′-nucleotide, but not adenine-3′-nucleotide), and certain nucleosidases. The high nucleolytic activity of the seminal plasma, particularly in man, bull, and ram, is reflected in the accumulation in it of split products, such as uric acid. Human seminal plasma contains about 2 to 6 mg of uric acid per 100 ml, but in the secretion of the bull seminal glands ("vesicles"), the concentration of uric acid is of the order of 10 to 30 mg per 100 ml, and in the ram seminal plasma, it varies from 4 to 23 mg per 100 ml. E. Leone in Italy has shown that, in the bull, uric acid is formed by the enzymic oxidation of hypoxanthine and xanthine; both these purines have been identified in the glandular tissue of bull seminal vesicles. These findings are interesting since in other organs of the bull and ram, unlike in man, purine metabolism does not terminate in uric acid. It could be that the various nucleotide-splitting enzymes, in which the seminal plasma abounds, aid the disposal of breakdown products of sperm nucleic acid arising from aged or otherwise damaged spermatozoa, tending at the same time to eliminate pharmacologically active purine and pyrimidine derivatives.

NITROGENOUS BASES

In addition to purines, there are several other nitrogenous bases occurring as characteristic constituents in the male accessory organs and their secretions. The oldest known is spermine, $NH_2(CH_2)_3NH(CH_2)_4$-$NH(CH_2)_3NH_2$, a base secreted in particularly large quantities by the human prostate and forming in human semen, on standing, the crystalline, insoluble spermine phosphate. Closely related with spermine is spermidine, $NH_2(CH_2)_3NH(CH_2)_4NH_2$, also found in human semen. Both spermine and spermidine undergo oxidation by an enzyme present in the human semen. Early investigators of spermine thought that even in fresh semen the base occurs as a phosphate salt which, owing to poor solubility, settles out in the characteristically shaped crystals, first observed by van Leeuwenhoek about 300 years ago. In a fresh human ejaculate there is, however, very little inorganic phosphate, and recent studies favor the view that the formation of spermine phosphate occurs only after the ejaculation and is the outcome of an interesting reaction between spermine, contributed by the prostatic secretion, and phosphoric acid that accumulates gradually following the action of prostatic phosphatase on phosphorylcholine, and possibly some organic phosphorus compounds derived from secretions of the other accessory organs.

Phosphorylcholine, the source of phosphoric acid needed to form the phosphate of spermine, yields on dephosphorylation yet another product, free choline, $OH(CH_2)_2 \cdot N^+(CH_3)_3$. When compared with other tissues and body fluids, human semen ranks as one of the richest sources of choline, values exceeding 2 g per 100 ml of semen having been recorded. That explains why a positive "Florence reaction," used in forensic medicine for the detection of seminal stains on clothing and similar material, can be obtained with dried human semen even when diluted with 1,000 parts of water.

Phosphorylcholine is sometimes also encountered in animal semen, but in lower concentrations. In the bull, ram, boar, and stallion, the bulk of "bound choline" occurs in the semen not as phosphorylcholine, but in the form of glycerylphosphorylcholine, a compound which, unlike phosphorylcholine, resists the action of the prostatic phosphatase and does not yield free choline after ejaculation. However, there is some indication that in the secretions of the female reproductive tract of the ewe there may be an enzyme capable of splitting glycerylphosphorylcholine. The richest source of glycerylphosphorylcholine in the bull, ram, boar, and stallion is the epididymis. In this organ it is formed as a true secretory product of the epithelium to such an extent that, in the species mentioned, we find it feasible to base the assessment of the epididymal contribution to the makeup of semen upon an analysis of glycerylphosphorylcholine in the whole ejaculate. Like the concentration of most substances secreted by male accessory organs, the level of glycerylphosphorylcholine depends on androgenic stimuli; it is suppressed by castration, but stimulated by testosterone administration.

Choline and its derivatives belong to a group of pharmacologically active substances and it has been suggested that choline as such, or acetylcholine, of which there may be as much as 1 mg per ml of human seminal plasma, is responsible for at least a part of the smooth-muscle-stimulating activity of human semen. The experimental evidence for this concept is weak; it is more likely that the oxytocic activity of human seminal plasma is due to prostaglandin, and not to choline or its derivatives.

Another nitrogenous base to which the oxytocic activity of semen has been attributed is serotonin or 5-hydroxytryptamine, $C_7H_6NO(CH_2)_2NH_2$. My own investigation in 1960, in the Lalor Foundation Laboratory at the Marine Biological Laboratory, Woods Hole, Massachusetts, surprisingly revealed that the so-called clasper siphon, an accessory organ of the male spiny dogfish, *Squalus acanthias*, produces a secretion uncommonly rich in serotonin. The presence of as much as 7 percent of serotonin on the dry weight basis in that secretion, which forms part of the seminal plasma, led me to suggest that in this viviparous fish, at any rate, 5-hydroxytryptamine may play a part in the process of reproduction, either by facilitating the ejaculation of semen in the male, or perhaps by eliciting contractions of the reproductive tract in the female and thereby aiding sperm ascent in the oviducts. Serotonin, or a base related to it, has also been reported in much smaller concentrations in mammalian semen. However, we have been unable to confirm that claim.

But there is in mammalian semen, in some animals such as the boar, stallion, mole, and hedgehog, a substantial amount of another nitrogenous base, characterized, like serotonin, by a strong diazo reaction (with nitrite and sulfanilic acid). That base is ergothioneine, $C_9H_{15}N_3O_2S$, the betaine of thiolhistidine. Because of the properties of its thiol group, ergothioneine is a highly reducing compound, capable of counteracting effectively the action of oxidizing agents such as hydrogen peroxide that could otherwise quickly immobilize spermatozoa. Hydrogen peroxide can be actually produced, though in small concentrations, by certain enzymes present in semen such as the amino acid oxidase and amine oxidase.

In the boar, ergothioneine is formed by the seminal vesicles, and in the stallion by the ampullary glands. But in the hedgehog, with which I have been experimenting recently, ergothioneine is produced in one particular region of the *glandula prostatica* complex, namely, its cranial portion (or *gl. prostatica I*) which is homologous with the seminal vesicles of other mammals. Apart from ergothioneine (about 50 mg per 100 ml) secretions produced in various parts of the hedgehog's "*glandula prostatica*" are characterized by the presence of corpora amylacea (first studied chemically by Gowland Hopkins), as well as fructose, citric acid, phosphorylcholine, glycerylphosphorylcholine, and sialic acid (fig. 1).

FRUCTOSE, POLYOLS, AND CITRIC ACID

Fructose (the chief carbohydrate of semen) is generally present in the semen of those species possessing a seminal vesicle, *e.g.*, man, bull, ram, boar, guinea pig, but there are exceptions to that rule. In the stallion, for example, the glands designated anatomically as seminal vesicles produce very little fructose; they contain a certain amount of a closely related substance, namely, sorbitol. This hexitol is also present in the vesicular secretion of the boar, together with a much larger quantity of another polyol, *meso*inositol. The prostate gland is in general devoid of the ability to produce fructose. The human prostate secretes citric acid abundantly but does not form fructose. The latter is produced in man by the seminal vesicles and ampullae. Consequently, any obstruction at the level of the ductus ejaculatorius in man will prevent both fructose and spermatozoa from reaching the urethra; the seminal ejaculates of such men will be aspermic and fructose-free, but are likely to show abnormally high levels of citric acid, due to the presence of prostatic secretion undiluted by any of the other accessory gland secretions. This fact has served as a helpful guide in the clinical diagnosis of various pathological conditions, *e.g.*, in cases of congenital bilateral aplasia of the vasa deferentia, seminal vesicles, or the ejaculatory ducts.

There are other species, however, in which at least some parts of the male accessory apparatus designated as "prostate" secrete fructose, either alone, or with citric acid. In the rat, fructose is secreted by the dorsolateral prostate and the so-called anterior prostate, better known as the coagulating gland; whereas citric acid is produced mainly in the seminal vesicle proper and the ventral prostate. The relation between the enzymic systems, which are responsible for the production of fructose and citric acid in the rat accessory organs, will be described by Dr. B. W. Harding. Another species in which fructose and citric acid are secreted in separate organs is the hedgehog.

In the hedgehog, the so-called *glandula prostatica* is a complex organ composed of three distinct pairs of glandular structures (fig. 1). The cranial portion or *gl. prostatica I* is homologous with the seminal vesicles, produces fructose (about 500 mg per 100 ml), and also some glycerylphosphorylcholine and phosphorylcholine. Immediately below the *gl.*

prostatica I (seminal vesicles) lies the second pair of organs which correspond closely to the prostate proper of other species, and are usually called *gl. prostatica II* or "internal prostate"; this secretes citric acid (about 100 mg per 100 ml) and also phosphorylcholine. The third and most caudally located portion of the prostate-complex, called *gl. prostatica III* or "external prostate," lies outside the pelvis, but is connected with the urethral canal by means of special elongated ducts. While some anatomists regard the two lobes of "external prostate" as homologous with certain parts of the prostate proper in other mammals, others consider them as being analogous to Cowper's glands or bulbourethral glands. Our studies show that the "external prostate" chemically differs from the "internal prostate." Its secretion has no citric acid, but is, instead, rich in sialic acid, and thus resembles the bulbourethral glands of certain other animals such as the boar. However, it should be added that in addition to these bulbourethral glands, the hedgehog possesses yet another pair of glands, quite small, deeply embedded in the muscle of the bulbar portion of the urethra itself, which might be regarded, on account of their location, as analogous to the Cowper's glands proper of man.

The formation of fructose and citric acid in the male accessory organs is a hormone-dependent process. In the rabbit, the fructose level gradually declines in the ejaculate, reaching trace values at the end of 2 weeks after castration, but this postcastrate decrease can be prevented or reversed by the subcutaneous implantation of a 100 mg testosterone pellet. Analogous results were obtained with rats, guinea pigs, and large domestic animals. The close correlation existing between the processes of fructose and citric generation on one side, and the action of testosterone on the other, is strikingly illustrated by experiments with transplanted accessory organs. When working in collaboration with Dr. Price at Chicago University, we demonstrated that small fragments of rat coagulating gland, about 1 mg in weight, grafted under the skin of normal male hosts, grew well and after some weeks accumulated at least as much fructose as the intact coagulating glands of the host itself. But when grafts were made of the ventral prostate, citric acid was produced exclusively. After castration of the hosts, the transplants of both the coagulating gland and ventral prostate lost their ability to generate fructose and citric acid, respectively, but both processes could be restored by injecting the hosts with testosterone propionate. Perhaps the most significant observation made in the course of these experiments was that the growth of the transplants and their biochemical activity occurred not only in male, but also in intact or spayed female hosts, provided that the latter were adequately treated with testosterone.

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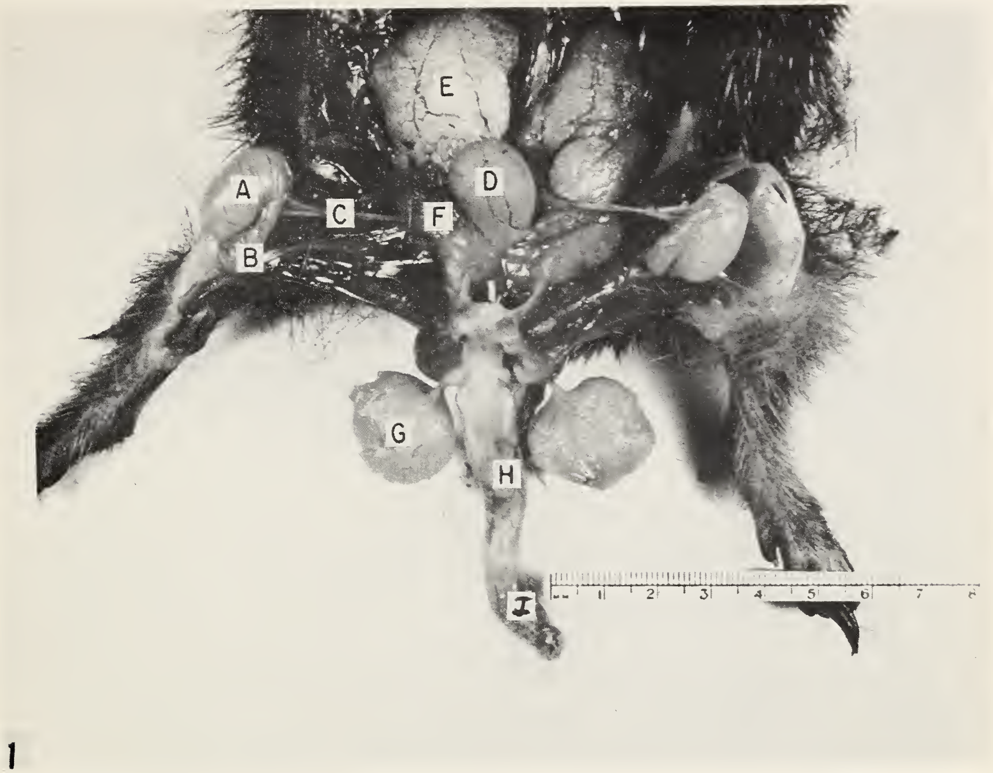
PLATES

PLATE 32

FIGURE 1.—The male reproductive tract of the hedgehog: *A*, testis; *B*, epididymis; *C*, seminal duct; *D*, bladder; *E*, seminal vesicle (*gl. prostatica I*), secreting ergothioneine, fructose, glycerylphosphorylcholine, and phosphorylcholine; *F*, internal prostate (*gl. prostatica II*), secreting citric acid; *G*, external prostate or bulbourethral gland (*gl. prostatica III*) secreting sialic acid; *H*, urethra, in the wall of which are located the Cowper's glands, distinct from external prostate; *I*, penis.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MANN

DISCUSSION

Dr. Williams-Ashman: I would like to say first that Dr. Mann's interesting report on the very large amount of sialic acid in the bulbourethral secretion of the boar emphasizes how important it would be to have some idea of understanding of the chemistry of the secretions of Cowper's glands and the glands of Littré in man, for those of us who are interested in the chemical physiology of the whole human semen. To my knowledge nothing is known about the composition of the urethral secretions or of Cowper's glands' secretions in man.

Second, as Dr. Mann's work has shown over the last 20 years, the secretions of the accessory glands and semen are a veritable cornucopia of chemical oddities. Dr. Mann has often written that there has been a marked tendency, a rather regrettable one, to read a function into every component of these secretions, and to insist that all these components in some way affect the well-being of the spermatozoa or affect the female genital tract and aid the transport of the spermatozoa to the site of fertilization. He indicated that although plasmalogen can be used as substrate for spermatozoa, a close derivative of it, namely, lysoplasmalogen, can be positively deleterious to the spermatozoa. Similarly, many years ago Zeller inferred that semen contains a very active diamine oxidase; this enzyme might oxidize spermine, and the recent work of the Tabors has shown that the oxidation products of spermine and other polyamines under the action of amine oxidase are extraordinarily toxic to spermatozoa. I wonder if Dr. Mann would comment on the possibility that the formation of products that are toxic to spermatozoa from precursors in the seminal plasma might be given more attention in relation to the problem of fertility.

Dr. Mann: I am unaware of any reliable information whether any of the potentially toxic products of sperm metabolism *in vitro* can also be formed and become efficacious in the female reproductive tract, *i.e.*, during the passage of sperm to the site of fertilization. The main toxic product of aerobic sperm metabolism, as studied *in vitro*, is hydrogen peroxide. I doubt if that reaction product would accumulate in the female tract, where oxygen tension is probably too low and sperm dilution with female secretions considerable; also there is catalase available from abundant leukocytes. As to lysoplasmalogen, if it were produced in a free form, it would become toxic to spermatozoa. But there is no evidence that lysoplasmalogen is actually liberated by respiring spermatozoa. On the contrary, our experiments indicate that it is retained by the spermatozoa in a protein-bound form. The possibility that spermine oxidation produces another toxic oxidation product in addition to hydrogen peroxide, presumably of organic nature, remains to be established.

There is no indication that the seminal plasma as a whole, or any one of its constituents, can influence the fertilizing potential of spermatozoa. This is in contrast to the strong evidence relating to the stimulatory influence seminal plasma has on sperm motility. The main function of the seminal plasma is to provide a suitable vehicle for spermatozoa. It is a mistake repeatedly made to attribute to every substance, produced in any one of the male accessory organs, a pivotal role in sperm survival or fertilization. Some of these substances may be end-products or by-products of the peculiar type of highly active metabolism characterizing the rapid turnover of proteins, lipides, carbohydrates, and other substances in the secretory epithelium of male accessory glands.

Dr. Bern: Dr. Mann questioned possible cell fragmentation and sloughing in the prostate and asked about corpora amylacea formation. At least in the rabbit prostate (Bern and Krichesky: Univ Calif Publ Zool 47: 175, 1943; Bern: Amer J Anat 84: 231, 1949) there is no doubt that whole cells and parts of cells contribute to the formation of corpora amylacea. This may represent nothing more than a by-product of cell turnover. I would like to ask anyone who has studied either rabbit semen or human semen about corpora amylacea. Are they visible as such as formed structures in the seminal fluid that is discharged?

Dr. Scott: What we call corpora amylacea are seen microscopically.

Dr. Farnsworth: I want to say a word about the citric acid problem. Citric acid is the principal secretory product of the rat ventral prostate and is elaborated in abundance by the prostates of most mammalian species studied. We previously demonstrated that the accumulation of citric acid represented an enhanced rate of biosynthesis rather than a depressed oxidation, and that the mobilization of acetate carbon for citric and fatty acid synthesis and of amino acids for protein synthesis was accelerated *in vitro* by the presence of 10^{-7} M testosterone. We postulated that the steroid could best do all this by making more adenosine triphosphate available to the system. We later reported at the American Cancer Society meeting in New York in 1960 that the rates of fatty acid and protein synthesis in prostate minces under nitrogen were quadrupled by addition of glucose or adenosine triphosphate, suggesting that glycolysis could well support these activities. It was found also that testosterone increased the glycolytic rate.

How could the androgen do this? We proposed that if the hormone could implement secretion of citric acid from the cell, or even the mitochondrion, it should favor citric synthesis. This would increase the demand for acetyl coenzyme A from fatty acid oxidation and glycolysis. We have, as mentioned, seen that glycolysis will support fatty acid and protein synthesis.

To test our hypothesis, we measured the effect of 10^{-7} M testosterone on citric acid synthesis by ventral prostate minces and the distribution of product. The data show increased production of radiocitric acid from both acetate-2-C^{14} and glucose-U-C^{14}. The hormone also increases the ratio of extracellular to intracellular citric acid, seemingly indicating a significantly stimulated secretion rate. We are not sure why the $C^{14}O_2$ is depressed by androgen in the acetate work and increased by the androgen with glucose. Possibly in the acetate experiments the CO_2 is lowered by secretory removal of citric acid from the Krebs cycle. With glucose, the extra CO_2 may come from the decarboxylation of pyruvate. We hope we have in this work a promising lead to the mechanism or site of action of androgen in the prostate.

Dr. Mann: I have been greatly interested in the work which Dr. Farnsworth has just summarized. It is particularly significant that androgens can stimulate so many diverse reactions in the prostate. There must be some common mechanism underlying all the varied effects, possibly some fundamental process linked with the function of nucleic acid and protein biosynthesis.

SOME ASPECTS OF FRUCTOSE AND CITRIC ACID SECRETION IN MALE ACCESSORY ORGANS OF REPRODUCTION[1]

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It has been established that the carbohydrate of mammalian semen is fructose. In man, ram, bull, boar, and certain other species, it is formed in the seminal vesicles or analogous organs; in the rat, the main fructose-producing organs are the coagulating gland and the dorsolateral prostate (*1*, *2*). Previous studies by Mann have shown that the biosynthesis of the seminal sugar is testosterone-dependent and involves the conversion of blood glucose into seminal fructose by the accessory organs (*3–6*). Hers (*7–9*) demonstrated that glucose was converted to fructose in ram seminal vesicle but via sorbitol rather than through phosphorylated hexose intermediates. The dehydrogenation and hydrogenation were catalyzed by two enzymes: 1) an aldose reductase (polyol-NADP oxidoreductase, EC 1.1.1.21),[5] which reduces glucose to sorbitol and depends on reduced nicotinamide-adenine dinucleotide phosphate (NADPH); 2) a ketose reductase, or so-called sorbitol dehydrogenase (L-iditol-NAD oxidoreductase, EC 1.1.1.14),[5] shown by Williams-Ashman and Banks (*10*) to be present in rat accessory organs, which oxidizes sorbitol to fructose and depends on oxidized nicotinamide-adenine dinucleotide (NAD^+). If the mechanism of fructose formation in male accessory organs depends on the presence of aldose reductase and ketose reductase, a correlation might be expected to exist between the production of fructose and the activities of these two enzymes. Consequently, Samuels, Harding, and Mann (*11*) undertook a study of the activities of aldose and ketose reductase and the actual rate of fructose formation in the glands of different animals.

Table 1 records the results of enzyme assays carried out on the four principal accessory organs of the rat, namely, the seminal vesicle, the coagulating gland, ventral prostate, and dorsolateral prostate. The results of fructose and citric acid analyses in the four organs are also given. It is clear that the ability of the different rat accessory organs to produce fructose coincides with high activities of both aldose reductase and ketose reductase but bears little direct relationship to that of ketose reductase alone. This dependence on the presence of both enzymes is borne out by the results obtained with the accessory organs of bull, ram, boar, and stallion (table 2). The bull and ram seminal vesicles, capable of producing large amounts of fructose, showed by far the highest aldose reductase activity and also the highest ketose reductase activity. Seminal vesicles of the boar, which produce much less fructose than those of the bull or the ram, had a much lower, though still distinguishable, aldose reductase activity. They lacked ketose reductase, however, and therefore would not be expected to be able to convert sorbitol into fructose efficiently. This is in accordance with the observations made by King and Mann (*14*) that the boar seminal plasma contains more sorbitol than fructose. Stallion seminal vesicle contained little of either enzyme; stallion seminal plasma was shown by King and Mann (*15*) to contain some sorbitol, but only a trace of fructose. Thus the correlation of the presence of both enzymes with the production of fructose, both in the same gland among species and between glands in the same species, indicates that fructose is probably synthesized in the male secondary sex glands primarily by the route postulated by Hers and Williams-Ashman.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

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[5] The nomenclature, polyol-NADP oxidoreductase, EC 1.1.1.21, and L-iditol-NAD oxidoreductase, EC 1.1.1.14, is according to the recommendation for nomenclature and coding of the Commission on Enzymes of the International Union of Biochemistry, 1961.

TABLE 1.—Activities of aldose reductase and ketose reductase, and concentrations of fructose and citric acid, in accessory organs of the male rat

	Activity (units/g of tissue)		Concentration (mg/100 g of tissue)	
	Aldose reductase	Ketose reductase	Fructose	Citric acid
Seminal vesicles	2. 7	39. 5	0	43
Coagulating glands	15. 2	27. 0	121	0
Ventral prostate	2. 8	1. 6	0	78
Dorsolateral prostate	10. 4	20. 4	47	56

The accessory organs were obtained from adult, male, albino rats treated for 1 week with testosterone propionate (1 mg per day per rat). The pooled glands from 12 animals were collected on dry ice, ground in a mortar with 0.15 M KCl and homogenized in ice-cold 0.15 M KCl in a Ten Broeck homogenizer. The homogenate was centrifuged at 4° C for 10 minutes at 15,000 × *g*. Fructose (*12*) and citric acid (*13*) were determined on aliquots of the supernatant. The remainder was subjected to fractional ammonium sulfate precipitation. After dialysis, the 0 to 50 and 50 to 70 percent saturated fractions were assayed for ketose reductase and aldose reductase by the methods previously described (*11*). A unit of activity corresponds to either the oxidation of 1 μmmole of NADPH per minute or the reduction of 1 μmmole NAD^+ per minute.

In bull, ram, and boar, the seminal vesicles produce both fructose and citric acid. In these species, however, the seminal vesicles have a rather complex cellular structure, and in the bull, for example, the vesicular epithelium contains three distinct types of cells, which probably have

Table 2.—Activities of aldose reductase and ketose reductase, and concentrations of fructose and citric acid, in the seminal vesicles of sexually mature mammals

		Activity (units/g of tissue)		Concentration (mg/100 g of tissue)	
	Number of animals	Aldose reductase	Ketose reductase	Fructose	Citric acid
Rat	5	0–2. 7	19. 4–39. 5	0–2	35–80
Bull	4	66–165	59. 0–136. 0	197–970	178–925
Ram	2	140, 191	385, 542	265, 293	45, 72
Boar	1	11	0. 5	35	110
Stallion	1	3. 5	5. 5	1	163

Seminal vesicles from the species indicated were collected on dry ice and analyses performed as described in table 1. The values shown are for the range observed or the total number of determinations. Units of enzyme activity are the same as in table 1.

different secretory functions (*16*). In the rat the secretion of fructose and citric acid occurs in separate organs. Also fructose and citric acid metabolism both depend on a common hydrogen carrier, NADP, but in different states of oxidation. In view of these considerations, it seems possible that these two processes may be mutually limiting when occurring in the same cell. If citric acid [as has been suggested by Talalay and Williams-Ashman (*17*)] is secreted because an unfavorably low ratio between $NADP^+$/NADPH prevents its further oxidation, it might be expected that reactions such as the reduction of glucose to sorbitol, which at the same time converts NADPH to $NADP^+$, might relieve this block to citrate oxidation and consequently prevent its accumulation and secretion.

To evaluate these considerations, Harding and Samuels (*18*) have examined in rat ventral prostate the activity and subcellular relationship to fructose and citrate metabolism of NADPH-cytochrome *c* reductase, the major NADPH-oxidizing system in other tissues. These studies are shown in table 3. It is apparent that the *in vitro* ability of isocitric dehydrogenase to generate NADPH is 3 to 4 times the potential of the cytochrome system to oxidize this substance.

In view of the similar subcellular distribution of the NADH and NADPH-cytochrome *c* reductase systems shown in table 3 and the well-known existence of phosphatases in rat ventral prostate, the possibility that the NADPH-cytochrome *c* reductase activity observed was due to the conversion of NADPH to NADH by a phosphatase or perhaps a transhydrogenase (*17*) was considered. Extensive studies of rat ventral prostate for evidence of a transhydrogenase were negative (*19*). However, when a crystalline alcohol dehydrogenase and acetaldehyde were incubated with the rat ventral prostate system reducing cytochrome *c*, no cytochrome *c* reduction was observed. When this same NADH-oxidizing system was incubated with either a purified NADPH-cytochrome *c* reductase or a purified glutathione reductase from rat liver, no alteration in activity of these enzymes was observed. Further, no

TABLE 3.—Intracellular distribution of NADPH-cytochrome *c* reductase, NADH-cytochrome *c* reductase, and isocitric dehydrogenase in rat ventral prostate

	NADPH-cytochrome *c* reductase	NADH-cytochrome *c* reductase	NADP-dependent isocitric dehydrogenase
Number of experiments	4	4	3
Fractions			
Absolute values (1+1S)	0.93 ± 0.22	8.36 ± 0.88	3.82 ± 0.66
Percentage			
1+1S	100	100	100
1	6	8	3
2	6	17	10
3	23	29	17
4	33	36	11
5	13	6	42
Recovery	81	96	82

Glands were collected from adult albino male rats treated with 1 mg testosterone per day for 1 week. The glands were expressed of secretions, weighed, and chilled in an ice-cold homogenizing medium consisting of 0.25 M sucrose-0.001 M Versene *p*H 7.4. The method for subcellular fractionation by differential centrifugation has been previously described (*19*). Fraction 1 consists largely of nuclei and cellular debris; fraction 2 of heavy mitochondria; fraction 3 of light mitochondria; fraction 4 of microsomes; fraction 5 is the 105,000 × *g* supernatant. "1S" indicates the supernatant from the first sediment prior to separation of the remaining particles and consequently (1 + 1S) is equivalent to the whole homogenate. Assays for the cytochrome *c* reductases have been described. NADP-specific isocitric dehydrogenase was determined by measuring the rate of reduction of NADP in the presence of 0.016 M nicotinamide, 6×10^{-4} M $MnCl_2$ and saturating levels of D-isocitrate and NADP. The absolute values are the mean and standard deviation of the mean for the number of experiments indicated and are expressed as units of activity per gram of tissue. One unit is equivalent to the amount of enzyme reducing 1 μmole of cytochrome *c* or NADP per minute at 25° C.

evidence could be found for the reaction of yeast alcohol dehydrogenase with NADPH from an examination of absorption and fluorescence spectra of NADPH in the presence of alcohol dehydrogenase. In addition, antimycin A inhibition curves for both of these systems in the mitochondria, light mitochondria, and microsomal fractions were all identical. In view of these findings it was concluded (*18*) that a specific NADPH-cytochrome *c* reductase in rat ventral prostate was either very low in concentration or absent.

Because of the importance of this observation to the understanding of citrate synthesis in ventral prostate and, if the same situation exists in other accessory reproductive organs, to fructose synthesis, an attempt was made to extract and purify the cytochrome *c* reductases from acetone powders of rat ventral prostate to verify the findings (table 4). Unlike rat liver cytochrome *c* reductases, it was not possible to purify the NADPH-cytochrome *c* reductase from acetone powders of rat ventral prostate by a combination of phosphate extractions, trypsin digestions, and ammonium sulfate precipitations. While a large percentage of the

NADPH-cytochrome *c* reductase activity in acetone powders could be extracted with salt solutions, this represented only 3 to 5 percent of the activity of the original tissue. However, it is obvious that in the first phosphate extract of the acetone powders a greater than tenfold relative increase in the activity of the NADPH-cytochrome *c* reductase over the NADH-cytochrome *c* reductase system was obtained. Even though the attempts to purify this system were unsuccessful, it is difficult to explain these results other than by the existence of both a NADPH- and a NADH-cytochrome *c* reductase in rat ventral prostate.

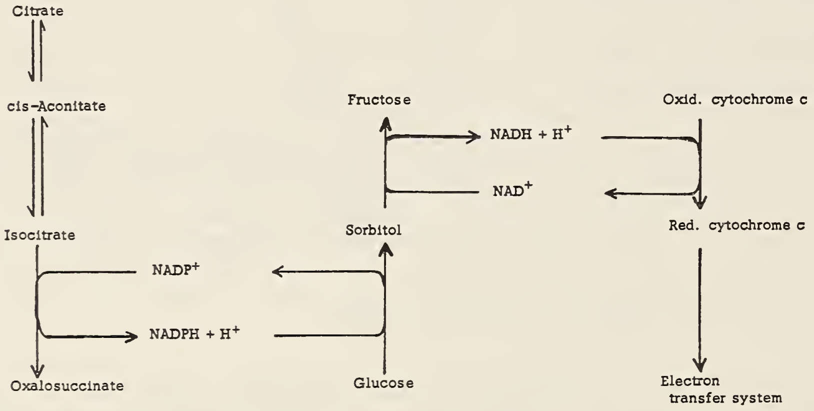
TABLE 4.—Rat ventral prostate NADPH and NADH-cytochrome *c* reductase preparation

	Milliunits per fraction		Milliunits per mg N	
	NADPH-cytochrome *c* reductase	NADH-cytochrome *c* reductase	NADPH-cytochrome *c* reductase	NADH-cytochrome *c* reductase
1) Tissue—whole homogenate	20, 200	182, 000	36. 3	327. 0
2) Phosphate extract of acetone powder	502	435	30. 2	26. 1
3) First $(NH_4)_2SO_4$ ppt from phosphate extract	62	137	4. 6	10. 0
4) Second $(NH_4)_2SO_4$ ppt from phosphate extract	0	4	—	—
5) Supernatant from 2d $(NH_4)_2SO_4$ ppt	90	80	—	—
6) Residue from phosphate extracts	825	3, 820	—	—
7) Other extracts of residue	246	3, 167	—	—

An acetone powder was prepared from 22.0 g of adult male rat ventral prostate. The powder was extracted with 0.1 M Na_2HPO_4 and the phosphate extract treated with trypsin followed by ammonium sulfate precipitation, according to the procedure of Haas *et al.* (*20*). The acetone powder was further successively extracted with 0.1 M Na_2HPO_4, 0.1 M Na_2HPO_4, and 0.3 percent deoxycholate [and 0.1 M Na_2HPO_4, 0.3 percent deoxycholate and 0.001 M Versene. The activity found in these extracts is all included in "other extracts of residue." One milliunit is equal to that amount of enzyme reducing 1 μmole cytochrome *c* per minute.

Returning to a consideration of the subcellular distribution of the enzyme shown in table 3, since presumably both isocitric dehydrogenase and aldose reductase are located in the soluble cytoplasmic fraction (*11*), while presumably the NADPH-cytochrome *c* reductase is in the particulate fractions, it is apparent that fructose synthesis may still exert an influence on citric acid secretion. This is possible because if the NADPH-cytochrome *c* reductase system in other accessory reproductive organs is particulate, as it is in ventral prostate and several other tissues (*21*, *22*), NADPH-cytochrome *c* reductase may be separated from a major portion of isocitrate oxidation occurring in the soluble supernatant fraction. Consequently, it is possible that the activity of aldose reductase may be limiting in the regulation of the $NADP^+/NADPH$ ratio.

In summary, aldose reductase and ketose reductase are both present when fructose is secreted by a variety of accessory reproductive organs of a number of different species. Either one or the other is absent in a number of tissues not having this capacity. These findings support the hypothesis presented by Hers that fructose synthesis occurs via a pathway involving sorbitol as an intermediate. Although NADPH-cytochrome *c* reductase has been shown to have the same subcellular distribution as NADH-cytochrome *c* reductase, and several other studies have suggested that a specific NADPH-cytochrome *c* reductase does not exist in rat ventral prostate, evidence has been presented here for its existence. However, this system has been shown to have a different subcellular distribution from that of isocitric dehydrogenase. More than 40 percent of the latter enzyme occurs in the soluble fraction, while the NADPH-oxidizing system is particulate. Since such a subcellular separation may prevent the cytochrome systems from accepting electrons and hydrogen from the cofactor involved in isocitrate oxidation, and since aldose reductase is a soluble enzyme which can catalyze the acceptance of such electrons and hydrogen by glucose, it has been postulated that this system may critically affect the ratio of NADP$^+$/NADPH and consequently regulate citrate synthesis. In text-figure 1 the manner in which these two systems, one producing fructose and the other metabolizing citrate, may be coupled to explain the inverse relationship between fructose and citrate secretion in accessory gland tissue is shown. In the absence of aldose reductase from a cell, no reduction of glucose to sorbitol with the accompanying oxidation of NADPH to NADP$^+$ can occur. Consequently, one might expect the rate of citrate oxidation to be low. But in a fructose-producing cell, where aldose reductase is present, NADP$^+$ would be regenerated in the reduction of glucose to sorbitol, thus facilitating the oxidative breakdown of citrate. Confirmation of this speculation must await a quantitative estimation of the oxidation or reduction of NADPH by all those systems utilizing this



TEXT-FIGURE 1.—Possible influence of fructose synthesis on citrate oxidation in male secondary sex organs where NADPH-cytochrome *c* reductase and aldose reductase are functionally separated.

cofactor (many of which have not been considered here) and possibly the demonstration *in vitro* of an inhibition of citrate synthesis by a fructose-synthesizing system.

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DISCUSSION

Dr. Samuels: In considering the work presented by Dr. Harding, it is important to distinguish between the question of the relationship between fructose and citrate and that of testosterone action. This is not, of course, discussing the work of Dr. Farnsworth in relation to testosterone action. That testosterone increases the biosynthesis of citrate is not contradictory to the work presented here. The point is that testosterone affects these enzyme systems as a whole.

We can see this in the following figures. For instance, one finds that not only does the aldose reductase change but also the ketose reductase increases with the maturity of the animal (table 1). When an animal was castrated, both activities were reduced and, when one steer was treated with testosterone, both increased (table 2). When one compares the effects of diet, both enzymes were influenced. Therefore, we must think of the influence of testosterone in terms of these enzymes being actually produced as a whole. The very fine work of Dr. Price and Dr. Williams-Ashman in the guinea pig shows that testosterone will not affect the production of citrate and fructose until there is a certain period of development, as though the mechanism necessary for the assembling of the enzymes first has to be unmasked before testosterone can have an effect. So it appears there are two problems here: (*a*) why is one compound and not the other produced in some cases, while in others there is mixed production; and (*b*) how does testosterone act on production? One is a proportionality factor and the other is an over-all effect.

TABLE 1.—Variation of enzyme activity with age in bull seminal vesicles

Age of bull	Aldose reduction (mμmoles/min/g)	Ketose reduction (mμmoles/min/g)
2.5 months	24. 4	5. 3
4.25 months	63	40
4.5 months	57	27. 5
1 year	66	100
6 years	128	136
18 years	52	61

TABLE 2.—Effect of castration, testosterone, and nutrition

	Aldose reduction (mμmoles/min/g)	Ketose reduction (mμmoles/min/g)	Testosterone administered
Steer A	6. 1	4. 2	0
Steer B, twin	33. 5	21. 5	31 days
Steer C, low calorie diet	50	23. 2	127 days
Steer D, twin; normal diet	128	54	127 days

Dr. Harding mentioned the relatively low aldose reductase activity compared with that of the TPN reducing enzyme, the isocitric dehydrogenase. We have to be careful here. One is measuring these reactions in different types of systems, and I am not at all sure that one can make a cross comparison between rates under these circumstances. The aldose reductase activity was measured under conditions which seemed to give maximum levels for the aldose reductase as separated; this does not necessarily mean that the rates measured thus can be compared directly with those of the isocitric dehydrogenase measured in the standard isocitric dehydrogenase assay. So, we want to be careful about overrating this comparison even though, as Dr. Harding has pointed out, the influence of fructose formation on citrate accumulation can be explained by the difference in the distributions within the cell.

Dr. Rosenkrantz: I would like to preface my question with a few remarks on analyses done on the dogs with prostatic fistulas. According to Dr. Harding's table, no work has been reported on the dog. It seems that the dog does not routinely produce fructose or glucose, and it is rare indeed when you can detect ribose by standard orcinol methods. Despite these facts, there is no doubt that carbohydrate is being elaborated by the dog prostate. Indeed, about 7 percent of the total dry weight can be accounted for as carbohydrate in a modified quantitative Molisch reaction. All this carbohydrate is retained by a dialyzing bag and seems to be associated in the form of a high molecular weight polysaccharide or as a glycoprotein. My question is, has any work been done along the line reported here on the dog?

Dr. Harding: We have not studied the isocitric dehydrogenase or the fructose synthesizing system in the dog. It has been shown, however, that the dog prostate secretes no fructose and very little glucose. We have studied the subcellular distribution of some of the enzymes in the dog prostate (Thesis: Subcellular fractionation and testosterone metabolism *in vivo* of rat ventral prostate and canine prostate. University of Utah, May, 1961, B. W. Harding) but extensive studies have not been pursued because of the difficulty in making a fractionation which can be compared to other tissues. I have nothing to offer regarding a nondialyzable carbohydrate.

Dr. Rosenkrantz: I would just like to add that this nondialyzable carbohydrate is hormone-sensitive. Upon treatment with diethylstilbestrol in these animals there is a significant fall in the carbohydrate level, and upon reintroduction of testosterone the "glycoprotein" is elevated again.

ANDROGEN REGULATION OF NUCLEIC ACID AND PROTEIN BIOSYNTHESIS IN THE PROSTATE [1,2,3]

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The past few years have revealed a close relationship between the nucleic acids and protein biosynthesis (*1*). The various steps in the conversion of amino acids to protein are mediated by specific nucleic acids (*1*). This report will be concerned with the role of the androgens in the regulation of nucleic acid and protein biosynthesis in the prostate.

NUCLEIC ACIDS

Effect of Castration

The well-known atrophy of the prostate after castration was accompanied by changes in the nucleic acids. The deoxyribonucleic acid (DNA) decreased in the mouse (text-fig. 1) (*2*) and rat [table 1, unpublished data (*3*)] at a lower rate than the organ.

TABLE 1.—Effect of castration and testosterone administration on the nucleic acids of the ventral prostate of the rat

		Prostate				
	Body weight (g)	Weight (mg)	Ribonucleic acid		Deoxyribonucleic acid	
			μg	μg/mg	μg	μg/mg
Normal	311	285	2,710	9.51	1,127	3.95
Castrated, 1 week	288	80	418	5.23	543	6.79
Castrated, 3 months	304	14	62	4.41	136	9.68
Castrated, treated 1 week with testosterone	291	173	1,223	7.07	444	2.57

Castration at 3 months of age (approximately 300 g body weight), testosterone (dose not given) injections were begun 1 month later when prostate weight was 15 mg (*3*).

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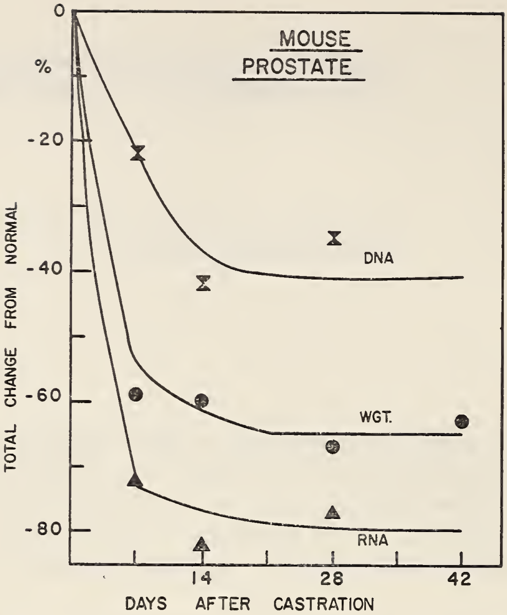
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] Supported by grant C-3566 from the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] Steroids used in this study were provided by Syntex, S.A., Mexico City, Mexico.



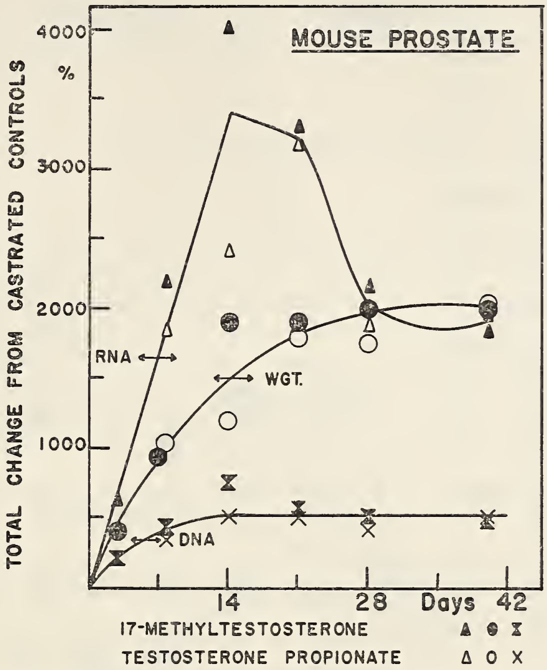
TEXT-FIGURE 1.—Effect of castration on the weight and nucleic acids of prostate of the mouse. Mice of the Holtzman-Swiss strain, 40 to 43 g body weight, were castrated at 100 to 110 days of age. There were 6 normal and 6 castrated mice at each time interval. Average values for 27 normal mice were: prostate weight, 55 mg; deoxyribonucleic acid (DNA), 2.82 mg per g; and ribonucleic acid (RNA), 3.38 mg per g (*2*).

The ribonucleic acid (RNA) in the mouse [(*2*), text-fig. 1], guinea pig (unpublished data), and rat [(*3*), table 1] decreased at a greater rate than the weight of the prostate.

Androgen Administration

17-Methyltestosterone, testosterone propionate (text-fig. 2), and testosterone produced similar patterns of response in the nucleic acids of the mouse prostate (*2*). The stimulation of growth of the tissue was accompanied by a restoration of the DNA to the normal level in about 5 days. The RNA, on the other hand, increased rapidly to above normal values but, with the attainment of maximum growth by the organ, the RNA then returned to approximately the normal value.

Androstan-17β-ol-3-one also was studied because its slower rate of absorption from the pellet provided a lower level of stimulation and also because its greater myotrophic than androgenic effect in the guinea pig is well recognized (*4*, *5*). The responses (text-fig. 3) at the higher dose level were essentially the same as those obtained with the testosterone compounds (text-fig. 2). At the lower dose level, the weight of the prostate attained a maximum value considerably below the normal value. The DNA, however, was restored to normal, and the RNA attained its normal level after 21 days and then decreased.



TEXT-FIGURE 2.—Effect of testosterone propionate and 17-methyltestosterone on nucleic acids and weight of the prostate of the castrated mouse. Mice were castrated at 4 weeks of age. A pellet of androgen was implanted subcutaneously 21 days after castration. There were 12 mice at each time interval, except at 21 and 40 days when there were 5 mice per group. The amount of steroid absorbed was: testosterone propionate 0.5, 1.4, 2.1, 3.1, 3.3, and 3.8 mg per period and 17-methyltestosterone 2.1, 5.0, 6.7, 9.0, 9.6, and 13.6 mg per period. Average values for 85 castrated control mice were: weight, 3.8 mg; deoxyribonucleic acid (DNA), 9.7 mg per g; and ribonucleic acid (RNA), 2.95 mg per g. Thirty-nine normal mice were studied at the same time. Average values were: weight 57 mg; DNA, 2.79 mg per g; and RNA, 3.37 mg per g. The results are plotted as total nucleic acids. Average total values for the normal mice are indicated by *horizontal arrows* (↔). Testosterone at 14 and 28 days produced essentially the same responses as methyltestosterone (*2*).

The castrated rat (table 1) responded to testosterone in a manner similar to that of the mouse (*3*). The total DNA was increased but at a slower rate than the weight of the organ. However, the RNA was increased at a greater rate [4] than the weight of the tissue.

The hypophysectomized-castrated rat (table 2) responded to androgen administration in practically an identical manner as the castrated rat (*4*). Furthermore, although growth hormone *per se* was ineffective, it enhanced the stimulatory action of the androgen. Extension of the studies in the rat would be of interest especially with respect to dose and duration of androgen administration. The studies just mentioned were at submaximal levels.

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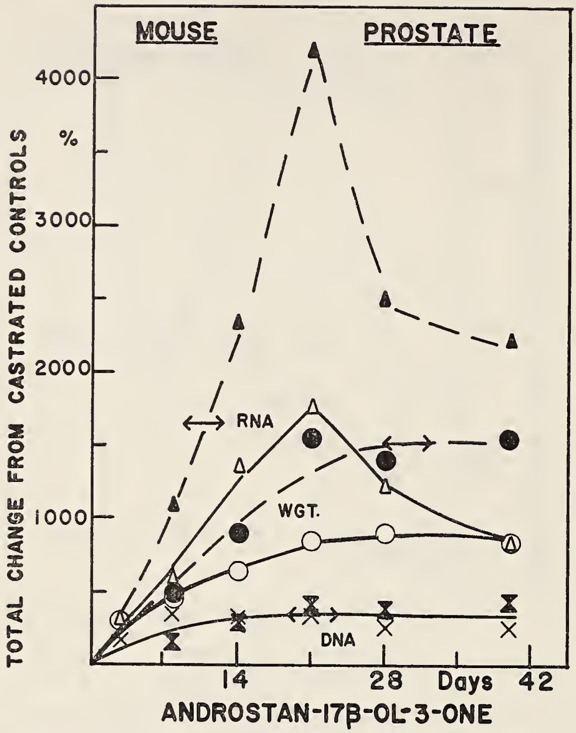
[4] The RNA of the seminal vesicles also increased at a greater rate than the weight after 3 days of androgen treatment, but after 10 days the concentration of the RNA had returned to the control level (*4*).



Text-Figure 3.—Effect of androstan-17β-ol-3-one on nucleic acids and weight of the mouse prostate. Castration was performed at 4 and 3 weeks of age for two series of experiments. Androgen was implanted subcutaneously as a single pellet at 21 days (*open circles*) and as two pellets at 39 days (*closed circles*) after castration in the respective series. Amounts of steroid absorbed were: single pellet, 0.6, 0.8, 0.9, 1.6, 1.8 and 2.2 mg per period; two pellets, 1.0, 1.7, 2.8, 3.5, and 4.1 mg per period. There were 5 castrated and 5 treated mice at each time interval (*2*). *See* text-figure 2 for control values.

Table 2.—Effect of testosterone propionate and growth hormone on weight and nucleic acids of the ventral prostate of young hypophysectomized-castrated rats

	Body weight (g)	Prostate weight (mg)	Prostatic ribonucleic acid		Prostatic deoxyribonucleic acid	
			µg	µg/mg	µg	µg/mg
Control	137	15	56	3. 73	108	7. 20
		Testosterone propionate for 3 days				
Growth hormone	164	15	49	3. 26	113	7. 55
Testosterone propionate	142	26	145	5. 58	156	6. 00
Testosterone propionate plus growth hormone	174	38	231	6. 10	198	5. 22
		Testosterone propionate for 10 days				
Testosterone propionate	141	129	750	5. 81	432	3. 26
Testosterone propionate plus growth hormone	202	163	1, 125	6. 90	457	2. 80

Data are from A. J. Lostroh, *Endocrinology* 70: 747, 1962. Rats were hypophysectomized at 45 days and castrated on the following day. Injections of growth hormone, 100 µg per day, were begun on the day of castration and testosterone propionate injections, 300 µg per day, 15 days later.

The guinea pig prostate showed essentially the same response to testosterone and androstan-17β-ol-3-one administration as that of the mouse and rat (unpublished data).

PROTEIN BIOSYNTHESIS

In Vivo

The rate of incorporation of glycine-2-C^{14} in the prostate of the guinea pig after intraperitoneal injection was markedly decreased by castration and increased to greater than normal by testosterone administration (table 3). Ten mg per day of testosterone propionate for 21 days and glycine-2-C^{14} given 12 hours before autopsy yielded essentially the same results (*6*). Analysis of the protein indicated that the amino acid composition was not altered by castration and androgen administration (unpublished data) and that the changes in rate of incorporation were a reflection not only of the incorporation of glycine but also of its metabolic products—serine, glutamic acid, and aspartic acid (*6*).

TABLE 3.—Incorporation *in vivo* of glycine-2-C^{14} into the protein of the guinea pig prostate

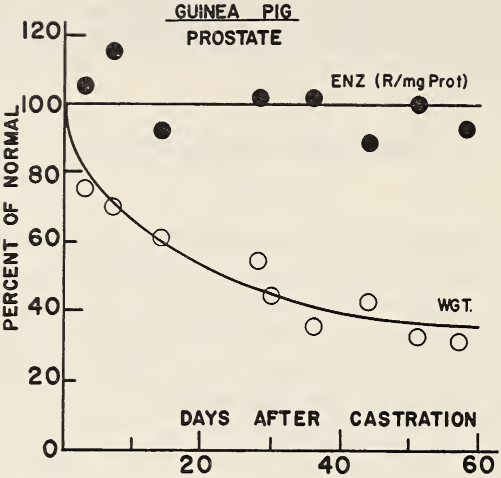
	Weight (mg)	Protein, (cpm/10 mg)
Normal	520	174
Castrated	220	78
Castrated, treated with testosterone, 14 days	430	306

Castration was performed at 468 to 538 g body weight. Testosterone was implanted subcutaneously as two 15 mg pellets 38 and 48 days later. Guinea pigs were killed after 14 days. Fifty μc glycine-2-C^{14} was injected intraperitoneally 4 hours before autopsy. Testosterone absorbed was 7.7 mg per 14 days. Tissues were weighed, immediately placed in 4 ml ice-cold 0.4 N $HClO_4$, homogenized, and the protein extracted. Values are the averages of 3 animals per group.

In Vitro

The first step in the mechanism of protein biosynthesis, activation of the amino acids, was not altered by castration (text-fig. 4) or androgen administration (text-fig. 5) (*7*). The rate of reaction remained constant. Thus, the total activity changed in direct proportion with the changes in weight of the tissue. Furthermore, the use of individual amino acids or groups of amino acids as substrate gave the same results (*7*).

The rate of incorporation of leucine-1-C^{14} into the protein of the prostate showed a small but not significant decrease after castration (table 4, unpublished data). The administration of testosterone (text-fig. 6) produced a variable and relatively small increase in the rate of incorporation of leucine-1-C^{14}, in spite of the fact that the organ was rapidly restored to its normal weight, but the activity in the seminal vesicle was markedly increased. Wilson reported (*8*) that androgen administration did not



Text-figure 4.—Effect of castration on adenosine triphosphate-pyrophosphate^{32} exchange and weight of prostate of guinea pigs. Castration was performed at 750 to 900 g body weight. Average values for prostate of 23 normal guinea pigs: weight 776 ± 27 mg and enzyme 32 ± 1.1 mμmoles pyrophosphate^{32} exchange per minute per mg protein (blank = 7.7 ± 0.1) (*7*).

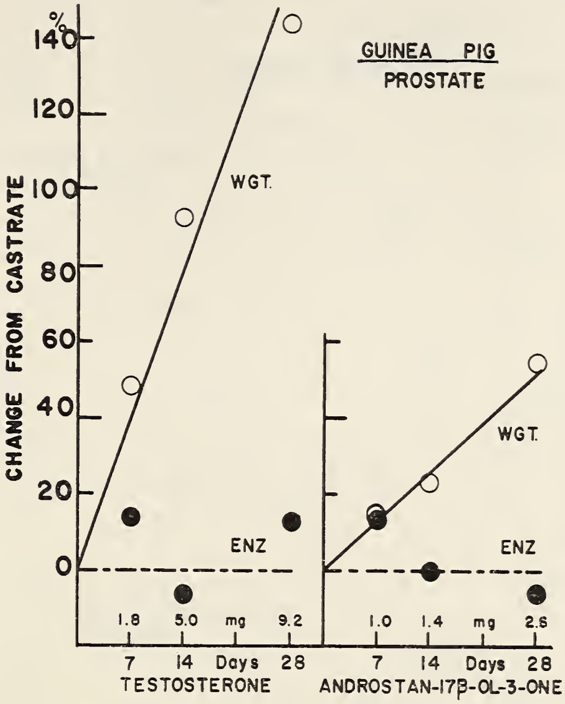
increase the rate of incorporation of amino acids in slices of prostate from young normal or castrated rats but had a profound effect on the rate in seminal vesicles.

Table 4.—Comparison of rate of incorporation of leucine-1-C^{14} into cytoplasmic protein (sedimented at 12,000 × *g*) of prostate of normal and castrated guinea pigs

Group	Number of animals	Prostate weight (mg)	Leucine-1-C^{14} incorporation (cpm/mg protein)
Normal	8	610	5. 7
Castrated	8	200	4. 8

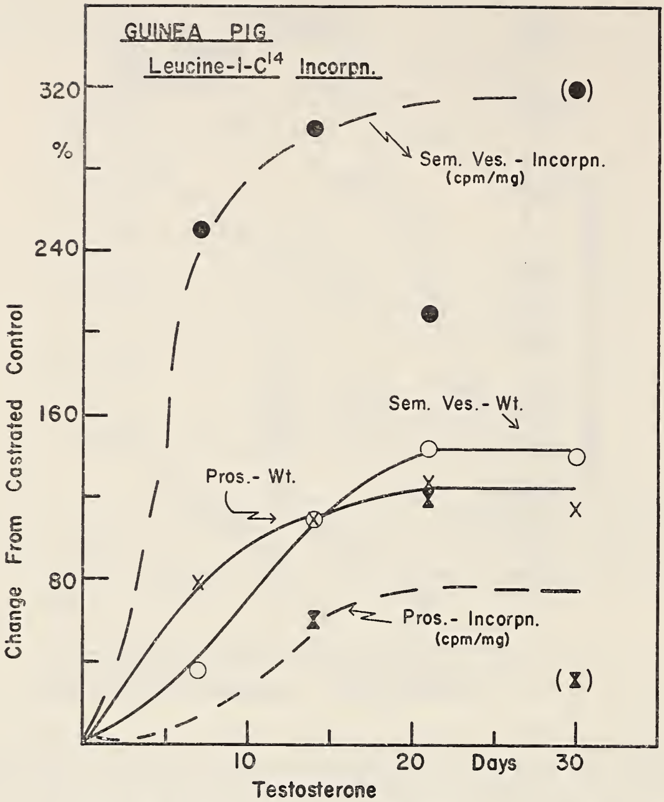
Guinea pigs were castrated at 640 to 870 g body weight. Experiments were performed 76 and 85 days later. Tissue was removed, chilled, chopped with scissors, and homogenized in 5 volumes of ice-cold medium containing 0.35 M sucrose, 0.01 M $MgCl_2$, 0.025 M KCl, and 0.05 M Tris buffer, *p*H 7.6. Homogenate was centrifuged at 12,000 × *g* for 10 minutes, and the supernatant fraction was used as enzyme. The reaction mixture contained: 0.2 μmole (0.8 μc) DL-leucine-1-C^{14}, 1 μmole adenosine triphosphate, 0.2 μmole guanosine triphosphate, 20 μmoles sodium phosphocreatine (from Sigma Chemical Corp., St. Louis, Mo.), 40 μg phosphocreatine kinase (Sigma, "21 μM units per mg at 30°"), and 0.5 ml enzyme. Reactants were prepared in homogenizing medium except DL-leucine-1-C^{14}, which was dissolved in water. Final volume of the reaction mixture was 1.0 ml. Incubation was for 30 minutes at 37°. The reaction was terminated by the addition of 4 ml of 0.4 N $HClo_4$.

To determine whether all of the protein biosynthesis activity was separated into the supernatant fraction by the customarily used centrifugal force of 12,000 × *g* for 10 minutes, the activity was also determined



Text-figure 5.—Effect of testosterone and androstan-17β-ol-3-one on adenosine triphosphate-pyrophosphate^{32} exchange and weight of prostate of castrated guinea pig. Male guinea pigs were castrated at 470 to 630 g body weight. Fifty-eight days later two pellets of testosterone (approximately 15 mg each) were implanted subcutaneously. Testosterone absorbed was: 4.8, 8.9, 12.0, and 14.7 mg per period. Average values for prostate of 12 castrated controls: weight 367 ± 10 mg and enzyme 36 ± 2.0 mμmoles pyrophosphate^{32} exchange per minute per mg protein (blank = 7.3 ± 0.4).

in the sediment. The rate of incorporation of leucine-1-C^{14} was found to be twice as great in the sediment as in the supernatant fraction. Therefore, in one experiment (table 5, text-fig. 6), the homogenate was centrifuged at only 3000 × *g*. The sediment still contained almost twice the specific activity of the supernatant fraction in the prostate of both the normal and testosterone-treated guinea pigs. Furthermore, the androgen produced a similar increase (30 and 38%) in the rate of incorporation of the amino acid in both fractions. The high activity in the sediment probably is due to the incomplete separation of the microsomal material. Harding and Samuels (*9*) reported that the preparation of rat prostate homogenate by the usual procedure resulted in incomplete separation of microsomal material from the nuclear and mitochondrial fractions by centrifugation. RNA, nitrogen, and phosphorus analyses of the particulate fractions of the guinea pig prostate (unpublished data) also indicated that considerable amounts of microsomal material had remained in the sediment.



Text-figure 6.—Effect of administration of testosterone on rate of incorporation *in vitro* of leucine-1-C^{14} into accessory sex tissues of the guinea pig. Experimental details as in table 4. Testosterone was implanted as two 15 mg pellets subcutaneously 76 days after castration. Testosterone absorbed was: 6.6, 10.3, 17.4, and 16.5 mg per period. Homogenate of the 30-day experiment was centrifuged at 3000 × *g* (*see* table 5).

Table 5.—Comparison of the rate of incorporation of leucine-1-C^{14} into protein of cellular fractions of guinea pig prostate

	Fractions at 3000 × *g*	
	Supernatant (cpm/mg/protein)	Sediment
Castrated	5. 0	9. 0
Castrated, treated with testosterone	6. 5	12. 4

Experimental details as in table 4 except that centrifugation was at 3000 × *g* and sediment was resuspended in 0.25 M sucrose to original volume of homogenate. Testosterone treatment lasted for 30 days.

DISCUSSION

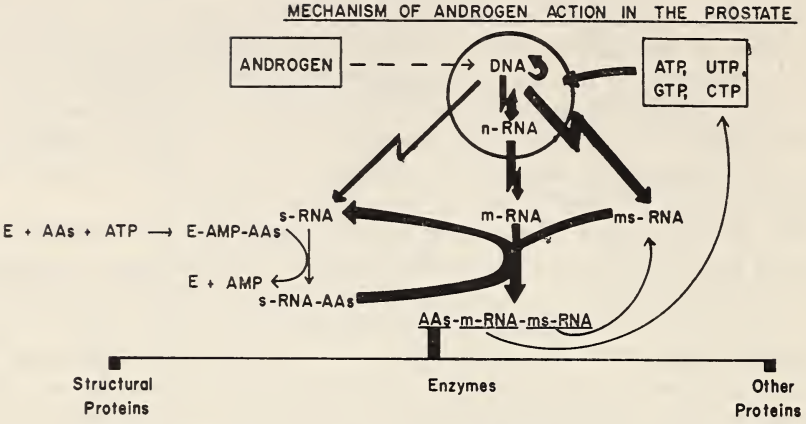
The regulation of nucleic acid and protein biosynthesis by androgens also has been studied in other target tissues. The response of the nucleic acids in the seminal vesicles (*2*, *10*) and kidney (*2*, *10*) of the mouse are very similar to those in the prostate (*2*). Furthermore, the changes in the RNA of the kidney after castration and androgen administration are due primarily to changes in the microsomal RNA (unpublished data). Marked changes in the microsomes of the seminal vesicles of testosterone-treated mice have been observed in studies with the electron microscope (*11*). Nucleic acid changes in the seminal vesicles of the rat (*12;* unpublished data) also are similar to those in the prostate.

The rate of protein biosynthesis in the prostate *in vivo* and *in vitro* is much lower than that of the seminal vesicles. Furthermore, androgens have a marked effect not only upon the activation of the amino acids (*7*) but also upon the incorporation of the amino acids into protein of the seminal vesicles (*6*). The prostate, on the other hand, does not seem to require androgen in the regulation of the activation of the amino acids and shows only a small dependence for amino acid incorporation into protein. This could mean that these systems in the prostate have different requirements or else entirely different systems are employed by the two tissues. It should be borne in mind, however, that the seminal vesicle is a more active tissue. It produces a copious secretion (approximately 2 times the organ weight) which is rich in protein (*6;* unpublished data). Thus, the detection of greater activity in the various steps of protein biosynthesis in the seminal vesicles is probably due to the more rapid rate of synthesis of the secretory protein. The absolute increase in weight of the two organs is not greatly different. The synthesis of the structural proteins probably occurs at a relatively lower rate and changes would be more difficult to detect.

The kidney of the mouse is dependent on androgen for protein biosynthesis (*13*). The rate of activation of the amino acids is not altered by castration or androgen administration. This reaction changes in direct proportion to the changes in weight of the kidney under the two conditions. But the rate of incorporation of amino acids into protein is reduced more than 50 percent by castration and increased to a maximum of approximately 900 percent on androgen administration. Furthermore, the rate of incorporation of amino acids into protein stops increasing after the kidney weight has attained its maximal increase in weight.

In the prostate as in the other tissues, the effect of androgen on the target organ is at least biphasic. First there is an acceleration of growth with its accompanying biochemical changes to a maximal level, and then a maintenance of this level which is accompanied by further changes in intermediary processes. The present evidence might be interpreted to indicate that the primary site of androgen action is at the nucleus (text-fig. 7) for the stimulation of the production of the ribonucleic acids essential for protein biosynthesis. Further potential support of this hypothesis is provided by the recent observation (*14*) that the RNA polymerase system of rat prostate nuclei is accelerated by the administration of testosterone.

The secondary phase, however, may vary in the different tissues. The available biochemical and enzymatic changes for the prostate and



Text-figure 7.—A working hypothesis for the mechanism of action of androgen in the prostate. DNA, deoxyribonucleic acid; RNA, ribonucleic acid; m-RNA, messenger RNA; n-RNA, nuclear RNA; ms-RNA, microsomal RNA; s-RNA, soluble RNA; AA, amino acid; E, enzyme; AMP, adenosine monophosphate; ATP, adenosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate.

seminal vesicle do not provide an explanation for this phase of androgen action (*15*, *16*). A working and reasonable hypothesis, however, can be formulated for the kidney. The arginase activity of the kidney is greatly increased by androgen administration. This increase, moreover, does not occur until the kidney has approached its maximal response in weight and then continues to increase while the weight of the kidney continues at the maximal level under the stimulus of the androgen. If we assume that arginase in the kidney is functioning in a cycle similar to that of the urea cycle of the liver, then the increase in arginase activity would place a greater demand on aspartic acid and carbamyl phosphate. Since these two substances are the starting material for the biosynthesis of the pyrimidines—uridine triphosphate and cytidine triphosphate—and the aspartic acid is also essential for purine synthesis, the increase in arginase activity would result in a diversion of these essential substances from the synthesis of uridine triphosphate and cytidine triphosphate and thus prevent the excessive formation of the ribonucleic acids essential for growth. The "alkaline" phosphatase of the kidney is also greatly decreased during the phase of maximum production of kidney weight by androgen stimulation.

This hypothesis might also apply to the accessory sex tissues. Small amounts of arginase are present in these tissues (*17*), but a detailed analysis of their response to androgen stimulation has not been made.

SUMMARY

Atrophy of the prostate after castration is accompanied by a small decrease in DNA, which is much smaller than the decrease in organ weight. Androgen administration restores the DNA to normal along with the

weight of the organ. The RNA of the prostate decreases after castration at a faster rate than the tissue weight. Administration of androgen restores RNA to the normal level. The more detailed studies in the mouse revealed that an accelerated rate of increase occurred during the period of stimulation of growth of the prostate to above normal levels but, as soon as the prostate attained its maximum weight response, the RNA began to return to the normal level.

The rate of incorporation of amino acids *in vivo* in the guinea pig was decreased after castration and increased above normal by androgen administration.

The amino acid-activating enzyme system of the prostate of the guinea pig changed in direct proportion to the changes in weight produced by castration and androgen administration. The amino acid-incorporating system showed a small but not statistically significant decrease in specific activity after castration, but showed a moderate variable increase after androgen administration at intervals from 5 to 30 days.

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EFFECTS OF CASTRATION ON MITOCHONDRIA OF RAT VENTRAL PROSTATE [1]

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The manner in which androgens exert their influence on specific target tissues is still largely unknown. An explanation has been sought partly in terms of direct effects on a variety of intracellular enzymes or substrate and coenzyme levels (*1*).

In the experiments to be described in this report, castration caused a consistent decrease in the numerical level of mitochondria in prostatic epithelial cells. This may well account for previously observed decreases in tissue concentration of a number of mitochondrial enzymes following castration. This view is supported by our additional observation that the enzyme content of isolated prostatic mitochondria is the same in castrated and intact rats.

MATERIALS AND METHODS

Homogenates.—Homogenates from rat ventral prostate were prepared as 10 percent weight by volume suspensions in 0.88 M sucrose or 0.85 percent NaCl with the use of Ten Broeck homogenizers.

Mitochondrial counts.—Mitochondrial counts were performed by the method of Shelton *et al.* (*2*).

Nuclear counts.—Nuclei were counted by the method of Weber and Cantero (*3*).

Enzyme assays.—We assayed succinate-cytochrome *c* dehydrogenase by measuring the rate of reduction of cytochrome *c* at 550 mμ in a Beckman Model DU spectrophotometer, using 1 ml capacity microcuvettes with a light path of 1 cm. To the cuvette were added (in order of addition) H_2O to 1 ml; KCN, 10 μmoles; phosphate buffer (K^+), *p*H 7.3, 60 μmoles; cytochrome *c*, 0.05 μmoles; K_2 succinate, 20 μmoles. Mixing after each addition was important. The reaction was started by the addition of enzyme, and the rate was taken from the linear portion of a plotted curve.

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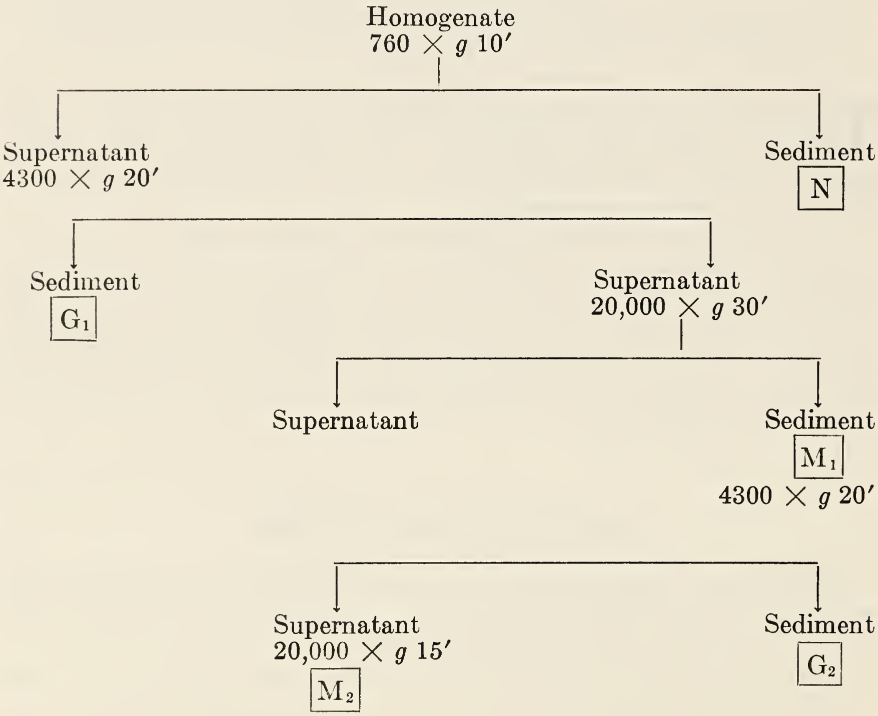
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] *Present address:* Department of Physiology, University of Cincinnati, Cincinnati, Ohio.

[3] Department of Urology.

Several levels of enzyme were used to insure that a linear relationship between enzyme concentration and rate existed. Results were expressed in terms of optical density change per minute. When the assay was done in this way, not only was it a measure of enzyme concentration, but also a test for the integrity of the mitochondrial membrane, as shown from the initial lag in the curve.

Preparation of mitochondria.—Isolation and purification were carried out as described for pancreas mitochondria (*4*). A summary of the procedure used to obtain the purified M_2 fraction is shown in text-figure 1.


Homogenate
760 × g 10′
Supernatant
4300 × g 20′
Sediment
N
Sediment
G1
Supernatant
20,000 × g 30′
Supernatant
Sediment
M1
4300 × g 20′
Supernatant
20,000 × g 15′
M2
Sediment
G2

TEXT-FIGURE 1.—Tissue fractionation in 0.88 M sucrose.

RESULTS

A decrease in the number of mitochondria and a decrease in the mitochondrial enzyme, succinate-cytochrome *c* dehydrogenase, were demonstrated (tables 1 and 2) in castrates. Purified mitochondria of castrates were as active as those of intact rats (table 3), which indicates that decrease of enzyme activity, as shown in table 2, was due to numerical decrease in mitochondria (table 1) and not to a decrease in activity of the mitochondria.

TABLE 1.—Mitochondria in homogenates of rat ventral prostate*

Endocrine state†	Protein (mg/100 mg wet weight)	Nuclei (No./100 mg wet weight × 10^{-6})	Mitochondria (No./100 mg wet weight × 10^{-10})	Mitochondria (No./nucleus)	Mitochondria (No./mg protein)
Intact (7)	15.2 ± 0.54	13.7 ± 1.3	0.86 ± 0.06	636 ± 24	5.82 ± 0.70
Castrated (8)‡	14.4 ± 1.1	13.9 ± 1.9	0.32 ± 0.05	229 ± 32	2.30 ± 0.38
P	Not significant	—	< 0.01	< 0.01	< 0.01

*Results are given in terms of mean ± standard error.

†Numbers in *parentheses* indicate number of individual experiments.

‡Four days after castration.

TABLE 2.—Changes in succinate-cytochrome *c* dehydrogenase in rat ventral prostate after castration

	Succinate-cytochrome *c* dehydrogenase units*				
	Per 100 mg wet weight		Per mg protein		Per single nucleus
Endocrine state†	Homogenate	Sediment‡	Homogenate	Sediment‡	Sediment‡ × 10^{-8}
Intact (7)	1.0 ± 0.2	1.1 ± 0.1	0.077 ± 0.014	0.20 ± 0.02	8.0 ± 0.44
Castrated (8)§	0.74 ± 0.2	0.66 ± 0.08	0.057 ± 0.009	0.10 ± 0.02	4.3 ± 0.43
P	> 0.1	< 0.01	> 0.1	0.001	< 0.01

*Results are given in terms of the mean ± standard error.

†Numbers in *parentheses* indicate number of individual experiments.

‡Homogenate was centrifuged at 25,000 × *g* for 1 hour. After washing with original volume of 0.88 M sucrose, it was resuspended in the original volume of 0.88 M sucrose.

§Four days after castration.

TABLE 3.—Specific activity of M_2 fraction from ventral prostate of intact and castrated rats

Fraction	Succinate-cytochrome *c* dehydrogenase (units/mg protein)	
	Intact	Castrated*
Experiment I		
Homogenate	0. 056(9)†	0. 030(9)†
M_2	0. 27	0. 37
Experiment II		
Homogenate	0. 040(5)	0. 018(16)
M_2	0. 36	0. 30
Experiment III		
Homogenate	0. 076(7)	0. 064(10)
M_2	0. 79	0. 76

*Five-day castrates were used in experiments I and III, and 4-day castrates in experiment II.

†Numbers in *parentheses* signify number of animals used. Ventral prostates were pooled in each group.

DISCUSSION

This study shows that there is a numerical decrease in mitochondria by about one half in the rat ventral prostate following castration. This probably accounts for the comparable decrease under similar conditions of such enzymes as succinoxidase, succinate-cytochrome *c* dehydrogenase, cytochrome oxidase, fumarase, aconitase, and malic dehydrogenase (*5–9*) all of which have been localized primarily in mitochondria (*10–15*), and also the decreases in the system incorporating C^{14}-acetate into fatty acids (*8*) which has recently been found localized in mitochondria (*16*). Other enzymes of the citric acid cycle such as triphosphopyridine nucleotide isocitrate dehydrogenase, which is located outside the mitochondria (*17*, *18*), have been shown not to decrease with castration (*6*).

Other extramitochondrial enzymes such as lactic dehydrogenase and glucose-6-phosphate dehydrogenase (*19*, *20*) either do not change or else increase following castration (*6*). An exception to this generalization is the enzyme, aldolase, which is primarily a soluble enzyme (*21*). Its tenfold decrease after castration (*22*) is, however, of an entirely different order of magnitude when compared to the decrease of the mitochondrial enzymes. Possibly a more direct hormonal effect may account for this phenomenon.

It is difficult to draw any conclusions from these experiments as to the precise location of succinate-cytochrome *c* dehydrogenase. In rat liver and ventral prostate this enzyme has been localized chiefly in heavy mitochondria (*12*, *23*).

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DISCUSSION

Dr. Kochakian: I would like to clarify Dr. Edelman's question regarding a discrepancy, between the lack of change in the nuclei after castration, in her experiments and our data. I should have been surprised if her results had been otherwise. Our studies in mice showed that after 4 days there was no or a very small decrease in the total deoxyribonucleic acid despite the considerable loss in weight of the prostate. Thus the loss of cells (hence nuclei) does not occur until after 4 days of castration. I would like again to make a plea for making both short and longer term studies.

Dr. Edelman: After 4 days we get considerable increase in the deoxyribonucleic acid concentration under the conditions that we use.

Dr. Hollander: I wonder if Dr. Edelman has any information on the decrease in mitochondrial mass that occurs following castration. Is it found within 12 hours?

Dr. Edelman: We have not done any experiments on this aspect. However, as far as the rapidity of the changes is concerned, we obtain a decrease in mitochondrial enzymes such as cytochrome oxidase within 24 hours.

Dr. Bern: One of the earliest cytologic manifestations of androgen deficiency in the ventral prostate is the disappearance of the so-called light areas within 4 days after castration (Moore, Price, and Gallagher, Amer J Anat 45: 71, 1930). These areas presumably represent the Golgi apparatus.

Dr. Harding: I would like to ask Dr. Edelman what changes occurred in the size-distribution of the mitochondria in the cells after castration. This may be of importance because the activity of the dehydrogenases and some of the other systems that were being examined would be dependent on the diffusion of a cofactor or substrate into the mitochondrial particle. It is well recognized that the pyridine nucleotides and certain dicarboxylic and tricarboxylic acids, for example, penetrate the intact mitochondrion poorly. One might expect that, if the size of the mitochondria were to decrease, the rate of diffusion of substrates into per unit mass of mitochondria would increase and consequently the concentration of an enzyme under consideration would appear to be greater. It is clear that under these circumstances changes in activity would be related to changes in permeability factors rather than to changes in enzyme concentration.

Dr. Edelman: From these experiments we could tell very little quantitatively about the comparative size of the mitochondria. Homogenization causes some breakage, but the mitochondria studied appeared to be intact.

Dr. Harkin: First, I would like to comment on the size of the mitochondria as the size of a mitochondrion can vary, *i.e.*, it can be swollen or shrunken without any change occurring either in the area of mitochondrial double membranes or the amount of potential mitochondrial enzymatic activity.

Secondly, changes occur in the supranuclear zone of prostatic epithelial cells after castration. A supranuclear clear zone in prostatic epithelium observed by light microscopy may or may not correspond to the Golgi complex observed by electron microscopy. In electron microscopic studies of rat ventral prostate, we found the Golgi zone of epithelial cells practically unchanged in size several weeks after castration.

Finally, changes following castration in the quantity of deoxyribonucleic acid per amount of prostatic mass may not be related entirely to changes in size of prostatic epithelial cells. Smooth muscle cells occupy a far greater percentage of total prostatic mass in the castrated than in the normal animal.

INCORPORATION OF AMINO ACIDS INTO PROTEIN BY CELL-FREE EXTRACTS OF THE PROSTATE GLAND: EFFECT OF TESTICULAR HORMONES AND POLYRIBONUCLEOTIDES[1, 2]

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This paper summarizes some recent studies on the incorporation of labeled amino acids into peptide linkage by cell-free extracts prepared from the rat prostate gland. The influence of testicular hormones and of polyribonucleotides on some of these reactions is discussed.

Perhaps the most spectacular advance in molecular biology over the last decade has been the demonstration that polynucleotides play a central role not only in the storage and replication of genetic information, but also in the expression of the genome in terms of cell structure and function. There is considerable evidence that most if not all the genetic instructions in mammalian cells (provided they are not infected with viruses) reside in the deoxyribonucleic acid (DNA) of the cell nucleus. This genetic information is almost certainly encoded as a linear sequence of nucleotide bases in the DNA, and is ultimately transcribed into the specific amino acid sequences of proteins. At present, it would be premature to discount completely the possibility that DNA may, under some circumstances, act as a direct template for assembly of activated amino acids into ordered peptide linkages (*1*). But there are many reasons to believe that intermediate ribonucleic acid (RNA) messengers usually carry genetic information from DNA to the ribonucleoprotein particles (ribosomes) which are a major site of protein biosynthesis. Such messenger RNA molecules appear to be synthesized by RNA polymerases, which catalyze a DNA-dependent copolymerization of nucleotides derived from ribonucleoside triphosphates, with the concomitant elimination of inorganic pyrophosphate (*2–6*). The nucleotide base composition (*7*) and almost certainly the base sequences (*8*) of the RNA's synthesized by this class of enzymes are complementary to those of the DNA primer, and do not seem to be dictated in any way by the origin of the enzyme protein itself. Although there are hints that messenger RNA molecules in certain bacterial (*9*) and mammalian (*10*) cells are metabolically unstable (and

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may be used as templates for synthesis of only a few protein molecules), in other biological situations the messenger RNA's may possess considerable stability, and probably serve their template function in a catalytic rather than a stoichiometric manner (*11*, *12*). This is borne out by the fact that actinomycin D, a potent and rather specific inhibitor of DNA-directed polyribonucleotide synthesis (*13–15*), can completely suppress the fabrication of RNA by some cells under conditions where protein synthesis is only partially inhibited (*14*, *15*). It must be emphasized that most of the RNA attached to ribosomes does not mirror the cellular DNA in its base composition (*16*); only a small fraction of the total ribosomal RNA probably serves a template function in protein synthesis. In some bacteria, messenger RNA's appear to attach themselves to various ribosomal particles to form "heavy" ribosomes which sediment faster than free ribosomes; such "heavy" ribosomes are active in protein synthesis, and seem to constitute only a small fraction ($<10\%$) of the total ribosomal population (*17*). The cytoplasmic ribosomes of many mammalian cells are studded along the membranes of the endoplasmic reticulum, and their physical properties and amino acid-incorporating activity have been studied extensively (*18–21*). Ribonucleoprotein particles are also present in mammalian cell nuclei (*22*).

Polyribonucleotides are also essential reactants in the biological activation of L-amino acids for protein synthesis. The soluble amino acid-activating enzymes (*23*, *24*) catalyze both the formation of L-amino acid adenylates as well as the transfer of amino acyl residues from the latter adenylates to the soluble transfer ribonucleic acids. The terminal base sequences of all transfer RNA's seem to be pCpCpA (*25*, *26*), and the activated amino acyl residues are linked to the 2′(3′) hydroxyl group of the terminal adenosine moiety (*27*). Transfer RNA's probably constitute a major proportion of the total soluble ribonucleic acid in cells. Until recently, it was believed that there existed a single and discrete transfer RNA (and amino acid-activating enzyme) specific for each of the 20 L-amino acids commonly found in proteins. However, there is now evidence that in bacteria and mammalian tissues, there may be more than one distinct transfer RNA for a given L-amino acid (*28*, *29*). McCully and Cantoni (*30*) have suggested that each individual amino acid-specific transfer RNA chain has a completely defined base sequence. At present, it is not known whether transfer ribonucleic acids are synthesized under the directive influence of DNA, *i.e.*, are gene determined, or whether the transfer RNA serves as its own template for its biosynthesis (*30*).

The functional differentiation of male accessory reproductive organs such as the prostate gland is utterly dependent upon testicular hormones (*31*, *32*). The growth and function of the prostate gland induced by testosterone and related steroids presumably must entail an increased rate of synthesis of many proteins (and particularly enzymes) which dictate the androgen-dependent secretory functions of this tissue. The instructions for the assembly of such proteins are, in all likelihood, already encoded in the DNA of prostatic cells of juvenile or orchiectomized animals and the proper reading of this genetic information only takes place if the gland is continually exposed to adequate levels of appropriate steroid hormones. It therefore seemed of interest to examine prostatic enzyme systems for the formation and utilization of ribonucleic acids that may serve a template function in protein synthesis. Previous experiments in this laboratory (*33*) delineated the properties of the DNA-dependent RNA polymerase in rat prostatic cell nuclei and the activity of soluble enzymes (present in both nuclear and cytoplasmic fractions) which catalyze the addition of cytidylate and adenylate residues to the ends of pre-existing sRNA chains (*34*). It has been known for some time that testicular hormones profoundly affect cytoplasmic basophilia in the prostate (*31*), this basophilia being abolished by treatment with ribonuclease. According to various authors, the cytoplasmic ribosomal population density in mouse seminal vesicle (*35*) and rat prostate (*36*) is not markedly decreased by castration or androgen treatment, although the levels of soluble and total RNA are greatly altered by such measures (*37*). Kochakian (*38*) reported that the activity of amino acid-activating enzymes in the rodent prostate hardly varies with the androgenic status of the host. In studies with isolated rat seminal vesicle slices, Wilson (*39*) showed that testosterone had little influence on the rate of penetration of labeled amino acids or on the synthesis of sRNA-amino acids, whereas the entry of labeled amino acids into protein was increased greatly by this hormone. Wilson concluded that testosterone primarily controls the transfer of amino acyl residues from sRNA-amino acids to microsomal ribonucleoprotein.

MATERIALS AND METHODS

Most of the experimental procedures and materials used are described by Liao and Williams-Ashman (*40*). We prepared DNA from rat liver and ventral prostate by a modification of the procedure of Dounce *et al.* (*41*), using tissue frozen in liquid nitrogen as starting material. The preparations of prostatic DNA were treated with ribonuclease as follows: The DNA obtained from the first dodecyl sulfate treatment was dissolved in 200 ml of 0.9 percent NaCl, and 0.05 mg of crystalline ribonuclease was added for each 10 ml of solution. After incubation at 35° C for 20 minutes, 1 volume of chloroform was added. The mixture was shaken and centrifuged at 5000 × *g* for 15 minutes at 25° C. The aqueous layer was pipetted off, and the chloroform treatment was repeated a further 3 times. The DNA was then precipitated from the aqueous solution by addition of 2 volumes of cold ethanol. The precipitate of prostatic DNA was washed with acetone and with ethanol, and finally dissolved in 0.01 M Tris-HCl buffer, *p*H 7.4, and 0.01 M NaCl to give a solution containing 1 to 2 mg DNA per 1 ml. The DNA solutions were dialyzed against the same buffered saline at 2° overnight. The solutions of rat liver DNA were dissolved in the same medium.

Approximately 200-fold purified RNA polymerase from *Escherichia coli* was generously donated by Dr. S. B. Weiss. The incorporation of cytidylate into RNA from CTP^{32}, labeled solely in the innermost (ester) phosphate, was determined by the method of Nakamoto and Weiss (*42*).

RESULTS

Activation of Amino Acids by Prostatic Enzymes

The generation of sRNA-amino acids was readily demonstrable with the amino acid-activating enzymes and sRNA present in "*p*H 5 precipitates" isolated from the soluble fraction of rat ventral prostate homogenates. Table 1 shows that such preparations catalyzed the conversion of L-valine-C^{14} to a product which resembled sRNA-valine-C^{14} in being (*a*) acid-insoluble in the cold, (*b*) rendered acid-soluble by brief exposure to hot dilute trichloroacetic acid, and (*c*) hydrolyzed by ribonuclease. A similar activation of L-leucine-C^{14} by such prostatic enzyme systems was observed. Prostatic sRNA was charged with L-valine-C^{14} and 19 other C^{12}-amino acids by methods previously used to charge rat liver sRNA (*40*). The ability of sRNA-amino acids, prepared from both prostatic and liver sRNA, to act as amino acyl donors to protein in reactions catalyzed by prostatic ribosomes was then compared side by side. Table 2 shows that the rate and extent of valine incorporation from labeled sRNA-valine were very similar with both the prostatic and hepatic sRNA-amino acid preparations. For this reason, experiments on the transfer of valine from sRNA-valine to protein by prostatic ribosomes were usually carried out with liver sRNA-amino acids, because large quantities of prostatic sRNA were not readily available.

TABLE 1.—Activation of L-valine by prostatic enzymes

System	Radioactivity incorporated into acid-insoluble fraction (cpm)
Complete	699
Omit ATP, phosphopyruvate, and pyruvic kinase	14
Add ribonuclease (0.2 mg)	34
Complete, hot TCA washing*	9

The complete system contained 50 μmoles Tris-HCl buffer, *p*H 7.8; 50 μmoles KCl; 10 μmoles $MgCl_2$; 2 μmoles ATP; 10 μmoles phosphopyruvate; 50 μg pyruvic kinase; 1.65 μmoles L-valine-C^{14} (3 mc/mmole) and rat ventral prostate *p*H 5 precipitate (3.9 mg protein). Incubated 20 minutes at 35°. Reaction terminated by addition of trichloroacetic acid, and acid-insoluble material prepared for counting as previously described (*40*), except that the exposure to *hot* trichloroacetic acid was avoided.

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*Acid-insoluble material treated with hot trichloroacetic acid (*40*) in order to render acid-soluble the valine-C^{14} bound to sRNA.

TABLE 2.—Prostatic and liver sRNA-amino acids as amino acyl donors to protein with prostatic ribosomes

Source of sRNA-valine-C^{14}	Specific radioactivity (cpm per mg sRNA)	Radioactivity incorporated into protein (cpm) after incubation for:	
		20 Minutes	60 Minutes
Prostate	3, 930	50	84
Liver	6, 840	119	156

Each vessel contained 0.51 mg of the appropriate sRNA labeled with L-valine-C^{14} and 19 other C^{12}-amino acids. Prostatic ribosomes prepared by method I (*40*), and equivalent to 2 g of fresh tissue, were added to each vessel. Other components of reaction mixture are described elsewhere (*40*). Incubated at 35° for the time intervals stated.

Incorporation of Free Amino Acids Into Protein

Free L-valine-C^{14} was incorporated into protein when it was incubated, in suitably fortified reaction mixtures, with both prostatic ribosomes and "*p*H 5 precipitates." A representative experiment is depicted in table 3. The incorporations were stimulated by guanosine triphosphate (GTP) and were almost totally dependent upon addition of adenosine triphosphate and an energy source (phosphopyruvate and pyruvic kinase). A soluble supernatant protein fraction was also required. If prostatic cell sap [obtained as the supernatant fluid resulting from sedimentation of prostatic microsomes (*40*)] was used as a source of soluble protein factor(s), then no further addition of "*p*H 5 precipitate" was necessary.

TABLE 3.—Incorporation of free L-valine-C^{14} into protein by prostatic ribosomes and amino acid-activating enzymes

System	Source of supernatant factor		Radioactivity incorporated into protein (cpm)
	Microsomal supernatant	*p*H 5 supernatant	
Complete	+	−	449
Complete	−	+	452
Omit ATP	+	−	321
Omit ATP, pyruvic kinase, and phosphopyruvate	+	−	65
Omit GTP	+	−	314
Omit ATP, GTP, pyruvic kinase, and phosphopyruvate	+	−	39
Omit *p*H 5 precipitate	+	−	466
Omit *p*H 5 precipitate	−	+	10

Each vessel contained the following ingredients (in μmoles unless otherwise stated) in a final volume of 1.0 ml: Tris-HCl buffer *p*H 7.8, 50; KCl, 50; $MgCl_2$, 10; phosphopyruvate, 10; pyruvic kinase, 0.05 mg; ATP, 0.5; GTP, 0.5; β-mercaptoethanol, 1; glutathione, 10; sucrose, 70; ventral prostate *p*H 5 precipitate (4.2 mg protein); prostatic ribosomes [prepared by method I (*40*)] equivalent to 2 g fresh tissue, and dialyzed supernatant protein (2 mg) from either microsomal or *p*H 5 supernatants; also added: 0.05 μmole each of L-valine-C^{14} (60 mc/mmole) and 19 other C^{12}-amino acids (*40*). Incubated 40 minutes at 35°.

This is because such "microsomal supernates" are replete with amino acid-activating enzymes for L-valine and other amino acids, and with sRNA. However, the soluble protein factor(s) was also present in the supernatant fluid obtained after centrifugation of the "*p*H 5 precipitate" following isoelectric precipitation of the cell sap fraction. When this "*p*H 5 supernatant" was used as a source of the soluble protein factor(s), the incorporation of free valine-C^{14} into protein required addition of amino acid-activating enzymes and sRNA, in the form of "*p*H 5 precipitate." This clearly demonstrates that the prostatic soluble protein factor(s) required for the incorporation of amino acids into protein is distinct from the amino acid-activating enzymes, as has been shown for the entry of amino acids into protein catalyzed by ribosomes from other animal sources (*43*).

Transfer of L-Valine-C^{14} From sRNA-Valine-C^{14} to Protein by Prostatic Ribosomes

To obviate complications inherent in the generation of sRNA-amino acids *in situ*, the transfer of amino acyl residues from sRNA-amino acids to protein by prostatic ribosomes was studied in systems to which sRNA-amino acids were added as such. In such experiments, liver sRNA was charged with L-valine-C^{14} and 19 other C^{12}-amino acids with the aid of rat liver amino acid-activating enzymes (*40*); the labeled sRNA-amino acids were then isolated and refined, and used as amino acyl donors with prostatic ribosomes. These ribonucleoprotein particles were prepared by treatment with deoxycholate of microsomal or other cell particulate fractions isolated from the rat ventral prostate gland. Some properties of this prostatic ribosomal amino acid-incorporating system have been documented in detail elsewhere (*40*). The incorporations were completely dependent upon the ribonucleoprotein particles, and maximal rates of reaction required the presence of 1) Mg^{++} ions, 2) a low molecular weight sulfhydryl compound (β-mercaptoethanol or reduced glutathione), 3) GTP, 4) a high energy phosphate generating system (phosphopyruvate and pyruvic kinase), and 5) soluble protein factor(s). The necessity for phosphopyruvate and its kinase is probably related to maintenance of adequate levels of GTP during the course of the reaction. Conditions were established under which the initial rate and final extent of transfer of valine from sRNA-valine to protein were proportional to the quantity of ribosomal material (as measured by the total amount of RNA attached to the particles) added to the reaction mixtures. Table 4 summarizes an experiment in which prostatic ribosomes were incubated with sRNA-valine-C^{14} for varying periods, at the end of which the ribosomes were reisolated from the reaction mixture by differential ultracentrifugation, prior to deproteinization and preparation of the ribosomal and soluble protein fractions for counting. It is evident that more than 90 percent of the total radioactivity transferred to protein remained firmly attached

Table 4.—Location of radioisotope incorporated from sRNA-valine-C^{14} into protein

Fraction measured	Radioactivity of protein fraction (cpm) after incubation for: 35 Minutes	90 Minutes
(a) Normal system		
Ribosomal protein	160	263
Supernatant protein	16	23
(b) In presence of testosterone (0.03 mM)		
Ribosomal protein	173	259
Supernatant protein	12	25

Components of reaction mixture were as previously described (*40*). Dialyzed supernatant protein (4.6 mg), and sRNA charged with L-valine-C^{14} and 19 other C^{12}-amino acids (0.38 mg; 1,990 cpm). One-tenth μmole each of free 19 C^{12}-L-amino acids other than L-valine (*40*) was also added. Testosterone added in 0.02 ml ethanol, which was added to the control vessels. After incubation for the times indicated, 12 ml of ice-cold medium B (*40*) was added, and ribosomes were reisolated by centrifugation at 100,000 × *g* for 60 minutes. One-ninth volume of 50 percent trichloroacetic acid was added to the supernatant fluid to precipitate the protein. Both the ribosomal and supernatant proteins were then processed for counting in the usual manner (*40*). Prostatic ribosomes prepared by method I (*40*), and equivalent to 2 g of fresh tissue were added to each vessel. Incubated at 35°.

to the ribosomal particles. Apparently, very little "peeling off" of ribosomal protein containing bound radioactivity occurs under these experimental conditions.

It was found that the rate and extent of amino acyl transfers catalyzed by prostatic ribosomes were markedly diminished with particles prepared from sexually mature animals that had been orchiectomized 2 to 3 days previously (*40*). This effect of withdrawal of testicular hormones was entirely reversed by treatment of the castrated animals with testosterone, but not by addition of this hormone to the isolated enzyme systems. Such experiments were conducted under conditions where the soluble protein transferring factor(s) added to all vessels was obtained from the prostates of the testosterone-treated castrates, and where the levels of these proteins were not rate-limiting. Attempts to enhance the amino acid-incorporating activity of prostatic ribosomes from castrated or androgen-treated animals by addition of a variety of naturally occurring polynucleotides were entirely unsuccessful (*40*). Similarly, no increase in valine incorporation by prostatic ribosomes was found upon addition to the reaction mixtures of highly purified RNA polymerase from *E. coli*, ATP, UTP, GTP and CTP, and prostatic DNA. Table 5 shows that the prostatic DNA readily served as a primer for the copolymerization of nucleotides from ribonucleoside triphosphates by the bacterial enzyme, in the same way as DNA isolated from rat liver or calf thymus. Wood and Berg (*44*) have shown that amino acid incorporation by bacterial enzymes is markedly enhanced by addition of RNA polymerase, the 4 ribonucleoside triphosphates, and DNA's from various sources (including animal tissues). The stimulation of amino acid incorporation was particularly large when DNA from T2 phage was employed. However, if heated T2 phage DNA was substituted for the native material, the stimulation of amino acid incorporation was almost abolished, whereas the ability of the heated phage DNA to serve as a primer for RNA synthesis was nearly as great as that of the unheated material. It is possible that only double-stranded DNA can serve as a primer for the RNA polymerase-catalyzed synthesis of RNA which can function as a template in protein biosynthesis. Little significance can be attached, therefore, to the apparent inability of prostatic DNA to prime the formation of active messenger RNA in experiments in which *E. coli* RNA polymerase was added to the prostatic ribosomal amino acid-incorporating system.

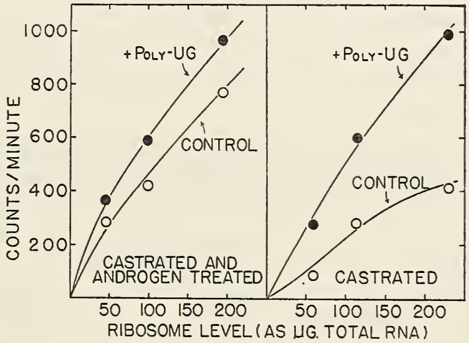
TABLE 5.—Priming of *Escherichia coli* RNA polymerase by prostatic DNA

Source of DNA	System	Radioactivity incorporated into RNA (cpm) from ester-labeled CTP^{32}
Calf thymus (0.1 mg)	Complete	13, 590
Calf thymus (0.1 mg)	Omit ATP, UTP, GTP	199
Rat ventral prostate (0.08 mg)	Complete	4, 245
Rat ventral prostate (0.08 mg)	Omit ATP, UTP, GTP	272
Rat liver (0.1 mg)	Complete	2, 574
Rat liver (0.1 mg)	Omit ATP, UTP, GTP	543

Each vessel contained 50 μmoles Tris-HCl buffer *p*H 7.4; 0.13 μmole ester-labeled CTP^{32} (10^7 cpm/μmole); 0.3 μmole each of ATP, UTP, and GTP; 1.5 μmole $MnCl_2$; 0.4 μmole spermidine and purified *E. coli* RNA polymerase in a total volume of 0.5 ml. Incubated at 35° for 12 minutes.

The failure of various natural polyribonucleotides to increase the transfer of valine from sRNA-valine to protein by prostatic ribosomes prompted further studies with synthetic polyribonucleotides. The polymers were prepared by the action of the polynucleotide phosphorylase of *Micrococcus lysodeikticus* on the corresponding ribonucleoside diphosphates. Experiments in the laboratories of Ochoa (*45*) and Nirenberg (*46*) have shown that poly U stimulates the incorporation of L-phenylalanine into peptide linkage in the presence of bacterial ribosomes, and that various heteropolymers enhance the incorporation of other L-amino acids. In particular, the entry of valine into peptide linkage was increased by samples of poly UG with high U/G base ratios. Jukes (*47*) has marshalled evidence to suggest that the triplet coding unit for L-valine is UUG. In experiments described in detail elsewhere (*40*), it was found that the transfer of valine from sRNA-valine to protein by prostatic ribosomes from recently castrated adult rats was increased as much as threefold by poly UG (U/G = 6.7). However, similar levels of poly UG had but little influence on valine incorporation by prostatic ribosomes prepared from testosterone-treated castrates (text-fig. 1).

The incorporation of valine was strongly inhibited by low levels of Puromycin in the absence or presence of poly UG. The stimulatory effect of poly UG was not mimicked by equivalent amounts of poly U, which accords with the known coding properties of the respective synthetic polyribonucleotides. The *in vitro* addition of testosterone (10^{-5} M) failed to influence the transfer of valine from sRNA-valine to protein by any of the prostatic ribosomal preparations, and did not affect the response to poly UG (*40*). A possible interpretation of such experiments with "artificial" messenger polyribonucleotides is that, under the stated conditions, prostatic ribosomes isolated from sexually mature rats that had been castrated 2 to 3 days previously are relatively deficient in template (messenger) RNA. Such template RNA presumably constitutes only a small fraction of the total RNA attached to the prostatic ribonucleoprotein particles.



Text-figure 1.—Effect of poly UG (U/G = 6.7) on valine incorporation by prostatic ribosomes from castrated rats, with or without treatment of the animals with testosterone. Animals killed 72 hours after surgery. Testosterone propionate (2 mg) injected into one group just prior to orchiectomy, and also at 27 and 54 hours thereafter. Prostate ribosomes isolated from pooled tissue obtained from 20 castrates or from 10 testosterone-treated castrates. Each vessel contained 2.2 mg dialyzed supernatant protein from prostates of testosterone-treated castrates, and 0.35 mg sRNA charged with L-valine-C^{14} and 19 other C^{12}-amino acids (19,250 cpm). Poly UG (if added), 48 μg. Ordinate: radioactivity incorporated into protein fraction. Abscissa: quantity of ribosomal material added (expressed as micrograms of total RNA attached to the particles). Other experimental details are given by Liao and Williams-Ashman (*40*), from which this data are taken.

Enzymatic Incorporation of Ribonucleoside Triphosphates Into RNA in Prostate Gland

Experiments in this laboratory (*33, 34*) have shown that the ventral prostate of the rat contains 2 distinct enzyme systems that catalyze the incorporation of nucleotides derived from ribonucleoside triphosphates into RNA. Table 6 summarizes the properties of these enzyme systems: 1) an RNA polymerase in cell nuclei which has many properties in common with DNA-dependent RNA polymerases described in other tissues and 2) enzymes associated with both nuclear and cytoplasmic fractions which catalyze the terminal addition of cytidylate and adenylate residues to sRNA chains. Despite repeated searches, it was not possible to detect any *cytoplasmic* enzyme systems in the rat ventral prostate which catalyze any extensive copolymerization of ribonucleoside triphosphates into the interpolynucleotide linkages of RNA (*48*), as opposed to terminal addition reactions. Crude nuclear enzyme preparations isolated from the ventral prostates of adult rats that had been orchiectomized 4 to 5 days previously exhibited a diminished ability to incorporate CTP^{32} (labeled solely in the ester phosphate) into RNA in reactions requiring the simultaneous presence of the other 3 ribonucleoside triphosphates (*33*). However, it is not known whether this effect of androgen withdrawal reflects any alteration in the levels of the prostatic RNA polymerase, or results from secondary changes, such as a decrease in the levels or priming ability of the DNA bound to the preparations, or changes in the activity of contaminating deoxyribonucleases or ribonucleases. Analysis of these findings has been hampered by our inability, as yet, to rid the prostatic nuclear enzyme preparations of bound DNA in such a manner as to retain RNA polymerase activity which is stimulable by exogenous polydeoxyribonucleotides.

TABLE 6.—Properties of enzyme systems in rat ventral prostate catalyzing incorporation of nucleotides from ribonucleoside triphosphates into RNA

Properties	RNA Polymerase	Terminal nucleotide addition reactions (pCpCpA)
Intracellular location	Nucleus	Nucleus and cytoplasm
Requirement for all 4 ribonucleoside triphosphates	Absolute	Nil
Inhibition by deoxyribonuclease	+++	Nil
Requirement for sRNA	Nil	Absolute
Inhibition by actinomycin D	+++	Nil
Location of nucleotides incorporated into RNA	Interpolynucleotide	Terminal
Relative activity (micromicromoles nucleotide incorporated into RNA, 15 min/100 mg fresh tissue equivalent at 37°)	50–70 (CTP)	ATP = 2,000 CTP = 30,000

DISCUSSION

The present experiments are consistent with the view that prostatic ribosomes isolated from recently castrated adult rats are relatively deficient in template RNA. However, other interpretations are possible. For example, a greater breakdown of added poly UG by prostatic ribosomes obtained from testosterone-treated as compared with oil-injected castrates could account for the large differences in the response of the valine incorporations to poly UG by the two types of preparation. Such an explanation seems unlikely because prostatic ribosomes from testosterone-treated castrates incorporated valine to a greater extent in the absence of the synthetic polynucleotide. Moreover, little or no response of such particles to poly UG was found in experiments with wide ranges of polynucleotide concentration. But it must be emphasized that the relation of environmental conditions to the response of prostatic ribosomes to synthetic polyribonucleotides requires more thorough investigation. It is also possible that the ribosomes of prostatic tissue are heterogeneous with respect to their amino acid-incorporating activity and their response to exogenous messenger RNA's, and that such particle heterogeneity may depend upon the androgenic status of the host.

Wilson (*39*) has suggested that the effects of testosterone on protein synthesis in the seminal vesicle are primarily related to a stimulation by this hormone of some step(s) in the transfer of amino acids from sRNA to microsomal ribonucleoprotein. Our findings are in accord with this hypothesis. They further suggest that testosterone basically influences the levels of template RNA attached to the ribosomes, rather than other factors which may limit the rate of protein biosynthesis, such as the availability or utilization of GTP in the transfer of amino acyl residues, or the activity of ribosomal proteins which may catalyze the template RNA-directed assembly of amino acids into ordered peptide linkage. Some recent experiments of Nemer (*49*) may be significant in this regard. Nemer found that the increased ability of sea urchin egg ribosomes to incorporate amino acids into protein which occurs after fertilization is accompanied by a diminution in the response of the particles to synthetic polyribonucleotides. Thus, the incorporation of L-leucine-C^{14} by microsomes from unfertilized eggs was stimulated by poly UC to a much greater extent than was found with particles isolated from fertilized eggs at the 2-cell stage. Similar results were obtained with L-phenylalanine-C^{14}, with poly U as the artificial messenger polyribonucleotide. Nemer suggested that ribosomes prepared from unfertilized eggs may be relatively free from messenger RNA, but that after fertilization an increasing proportion of the ribosomes become pre-empted with new messenger RNA.

Methods have recently been described for the study of the formation of rapidly labeled (possibly messenger) RNA in animal cell nuclei (*50*), and also of the stimulatory influence of animal tissue RNA's on amino acid incorporation into protein by bacterial ribosomes (*51*). Experimental procedures of this nature may help to decide whether testosterone primarily influences the fabrication of messenger RNA, or rather the transport or attachment of messenger RNA to ribosomes in the prostate gland. If the locus of action of testosterone is indeed in this biochemical realm, it might be expected that the hormone basically influences the synthesis or utilization of messenger ribonucleic acids which carry information for the assembly of proteins concerned with the specialized functions of male accessory reproductive organs.

SUMMARY

Factors affecting the incorporation of labeled amino acids into protein by isolated prostatic ribonucleoprotein particles are described. Prostatic ribosomes isolated from adult rats which were castrated 2 to 3 days previously show a diminished ability to catalyze the transfer of valine from sRNA-valine to protein. This effect of withdrawal of testicular hormones was reversed by treatment of the orchiectomized animals with testosterone. The incorporation of valine by ribosomes from recently castrated animals was markedly increased by addition of the synthetic, randomly ordered polyribonucleotide poly UG. But ribosomes isolated from testosterone-treated castrates responded to poly UG to a much lesser extent. The experiments suggest that testosterone controls the levels of template RNA attached to isolated prostatic ribonucleoprotein particles. Enzymatic pathways for the incorporation of ribonucleoside triphosphates into RNA in the prostate gland are summarized.

* * *

Note added in proof: Since this article was submitted for publication, further experiments in this laboratory have strengthened certain of the conclusions given. In collaboration with D. A. Silverman, a study was made of factors affecting the transfer of radioisotope from added sRNA-phenylalanine-C^{14} to protein-like material by prostatic ribosomes. The incorporations were measured under conditions where they were proportional to the amount of ribosomal material added to the reaction mixtures, and where the levels of GTP and of mandatory soluble protein factors were not rate-limiting. The entry of phenylalanine into the protein fraction catalyzed by prostatic ribosomes from normal, sexually mature rats was stimulated from fivefold to 20-fold by addition of polyuridylic acid (poly U). Also, the incorporation of phenylalanine by prostatic ribosomes from adult rats which were orchiectomized 72 hours previously was compared with the incorporations by ribosomes from testosterone-treated castrates, the soluble proteins added being prepared from the prostates of the latter group, and added in excess. In the absence of poly U, the phenylalanine-incorporating activity of ribosomes from the castrates was less than 50 percent of that observed with prostatic ribosomes from androgen-treated castrates. But this difference was no longer apparent when the phenylalanyl transfers were determined in the presence of poly U, which stimulated the incorporations by ribosomes from the castrates to a greater extent than found with ribosomes from the testosterone-treated animals. These findings accord with experiments on the influence of testicular hormones on valine incorporation described in this paper.

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DISCUSSION

Dr. Edelman: I would like to ask Dr. Williams-Ashman if synthesis of RNA is affected by castration or testosterone; and if it is, could it not be the postulated primary site of hormone action?

Dr. Williams-Ashman: In previously published experiments (R. L. Hancock, R. F. Zelis, M. Shaw, and H. G. Williams-Ashman, Biochem Biophys Acta 55: 257, 1962), we found that "aggregate" enzyme preparations isolated from rat ventral prostatic cell nuclei readily catalyzed the incorporation of P^{32}-ester labeled CTP into the interpolynucleotide linkages of RNA, in reactions requiring the simultaneous presence of ATP, UTP, and GTP. It was observed that the incorporations (expressed in terms of enzyme activity per unit quantities of fresh weight of tissue) were depressed by a factor of more than 2 with preparations obtained from animals 5 days after orchiectomy. The activity of this enzyme system is strongly inhibited by deoxyribonuclease, and the prostatic enzyme resembles DNA-dependent RNA polymerases described in other animal tissues. However, I am loath to ascribe the castration-induced decreases in CTP incorporation to any direct effect on the RNA polymerase. Despite repeated efforts in our laboratory by Dr. L. Jurkowitz, we have been unable to remove DNA from the enzyme preparations under conditions in which RNA polymerase activity that is stimulable by exogenous DNA is retained. Also, we have not determined whether the enzyme preparations used in our studies exhibit ribonuclease activity, which may degrade some of the synthesized RNA. Thus the effects of testicular hormones on CTP incorporation into RNA could result from changes in the levels (or priming ability) of the bound DNA, or in the activity of any ribonuclease, associated with the aggregate enzyme preparations. Much further work will be required to determine whether testosterone primarily influences the biosynthesis of RNA, and especially of messenger RNA's, in the prostate gland.

Dr. Harding: Are the ribosomes and the messenger RNA only at fault in the castrated prostate? And have you done experiments which rule out the possibility that the amino acid-activating enzymes and possibly the soluble RNA are also decreased or ineffective?

Dr. Williams-Ashman: Our experiments on isolated prostatic ribosomes cannot overrule any possible effects of testosterone on the levels of either amino acid-activating enzymes or of sRNA in the prostate gland. These factors have been studied extensively by Dr. Kochakian and his co-workers (Amer J Physiol 210: 1068, 1961, and Endocrinology, 70: 99, 1962). However, in our ribosome experiments we added sRNA charged with L-valine-C^{14} and 19 other C^{12}-amino acids to the reaction mixtures as such, and they are therefore uncomplicated by the levels of sRNA, or the activity of amino acid-activating enzymes. Furthermore, the levels of soluble protein factors required for the incorporations were shown, in separate experiments, not to be rate-limiting. Under our experimental conditions, the rates of amino acid incorporation were limited by the quantity of ribosomes added to the reaction mixtures. Our experiments involving the addition of poly UG suggest that the levels of bound template RNA associated with the ribosomes determine the rate and extent of amino acid incorporation.

Dr. Mann: Have you done some experiments on the fate of testosterone, that is, on the problem whether testosterone is either taken up or in any way changed by either the ribosomes or the nuclear preparations?

Dr. Williams-Ashman: No, Dr. Mann, we have not. I am aware, of course, of the very interesting experiments carried out by you in collaboration with Drs. Harding and Samuels showing that the rat prostate converts testosterone to androstenedione, and that these steroids are associated with the microsomal fraction of this organ. In attempts to demonstrate effects of steroids *in vitro* on our prostatic ribosomal system, we have added mixtures of testosterone and androstenedione, as well as the individual steroids, both in the absence and presence of DPN and TPN. However, we have not observed any reproducible effects of such steroid additions on the amino acid incorporations.

Dr. Hollander: Your data show that the rate and the final extent of incorporation of the amino acid is different in the castrate and control. I was interested in the difference in extent rather than rate and the possibility that this would be interpreted as a limitation in binding site, possibly of messenger, as you suggest, but possibly also a binding for product, and that the additional messenger in some way achieves a solubilization of product. You showed that about 90 percent of product is bound, but did you also do this in the presence of the additional synthetic messenger?

Dr. Williams-Ashman: We have not examined the solubility of the labeled protein formed in the presence of added poly UG. However, in the absence of this synthetic polyribonucleotide, more than 90 percent of the labeled valine transferred from added sRNA-valine to the protein fraction remains firmly bound to the ribosomes when the reactions are run to completion.

STUDIES OF FIBRINOLYSIS AND ACID PHOSPHATASE IN CANCER OF THE PROSTATE[1, 2]

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In the last several years, workers in this laboratory have spent considerable time studying two enzymes from the human prostate: fibrinolysin and acid phosphatase. I should like to review briefly certain aspects of our earlier work on the prostatic fibrinolysin and of our more recent work on acid phosphatase. Although they constitute two different subjects, they have much in common. Both are characteristic of the prostatic glandular tissue in which unusually large amounts of these two enzymes are found. Their biological significance is not completely understood, though some information is now becoming available.

These two enzymes are also present in carcinoma of the prostate, and certain clinical manifestations observed in patients with cancer of the prostate can be explained by the presence of these enzymes in the extracellular fluid of the body. Finally, the level of these two enzymes in the blood of patients with carcinoma of the prostate is strongly influenced by sex hormones and therefore reflects the hormone dependency of this type of cancer.

I will begin with a few remarks concerning the prostatic fibrinolysis, the syndrome first described by us in 1952, consisting of hypofibrinogenemia and bleeding occurring in approximately 12 percent of patients with advanced cancer of the prostate, and explained by the release of a proteolytic enzyme from the prostatic tissue into the blood (*1*, *2*). We have demonstrated that there is actually such a proteolytic enzyme in the normal prostate and prostate cancer, and this enzyme can be identified in the blood of patients with this syndrome (*3*). Instances of this new syndrome have now been reported by a number of investigators. Since the original description, it has become well recognized as a complication during the course of prostatic cancer that assumes increased importance if any surgical manipulations are contemplated on these patients. However, for the clinician, the real interest of this recognition of the

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pathogenesis of bleeding, in cancer of the prostate, is twofold. First, it has practical interest since this recognition gave a logical orientation to the search for the therapeutic agent and turned attention to enzyme inhibitors. The trypsin inhibitor from soy bean was found an effective treatment for this type of hemorrhagic disease (*4*), but recently the use of ϵ-aminocaproic acid, proposed by Japanese investigators, has made the soy-bean polypeptide obsolete as a therapeutic agent. ϵ-Aminocaproic acid is an excellent inhibitor of the prostatic proteolytic enzyme, has little toxicity, and is easy to administer (*5*). Thus a really effective treatment of this hemorrhagic disease is now available.

A second point of interest is that the study of hemorrhage in prostatic cancer provided a good example of the concept that the release of a tissue constituent in the blood may produce important biochemical alterations in the organism and create a disease which, in this instance, is hemorrhagic. In 1952, this was a new concept; the existence of abnormal enzyme levels in certain diseases was certainly known, but, until the prostatic fibrinolysis was described, these elevated enzyme levels had diagnostic significance but had not been credited with producing specific pathologic manifestations. Thus prostatic fibrinolysis and hemorrhage represent one of the early observations or perhaps even the first observation of a specific disease caused by the release into the circulation of a constituent of cancer tissue. There have been several such instances discovered since, and the cardiovascular syndrome of patients with generalized carcinoid is a good example. However, the agent responsible for the production of the carcinoid syndrome is not an enzyme but is a potent pharmacologic agent activating certain physiological mechanisms in the organism. This is an entirely different process from the production of pathologic changes by the direct effect of enzymes originating from tumors on substrates available in the extracellular compartment of the body. The study of this latter process represents a promising research program for the future.

For those interested in the effect of hormones on the prostate and, particularly, cancer of the prostate, it should be mentioned that the level of prostatic fibrinolysin in the blood is influenced by the administration of male and female hormones. Estrogens decrease the level and, in one patient in whom it was tried, androgens increased the level (*3*). These effects are entirely similar to the hormone action on serum acid phosphatase.

These observations renew the old question: How do hormones produce these enzyme changes? The data show that in a few hours the administration of estrogens brings about a sharp decrease in the serum level of prostatic fibrinolysin and of acid phosphatase when these two values are elevated in patients with cancer of the prostate (*3*). This could occur, of course, through reduction in the amount of cancer tissue producing these enzymes, under the influence of the hormones, though the rapidity of the response is somewhat surprising. For instance, it seems unlikely that the administration of estrogens reducing the serum level of acid phosphatase by 50 percent in 48 hours does so by reducing the amount of prostatic cancer tissue by 50 percent. It is suggested that the reduction in enzyme activity is the primary effect of the hormone and testifies to a metabolic alteration subsequently producing inhibition of growth.

These considerations led us to attempt to study the effect of hormones on the production of acid phosphatase by prostatic tissue *in vitro*. However, we soon stopped our endeavor because of a certain action of the calcium present in the incubation mixture of the prostatic acid phosphatase. This calcium effect made it impossible to interpret the fluctuations of acid phosphatase activity in our experiments, and it had to be studied first.

In brief, it was found that calcium ions reversibly inactivate the acid phosphatase of the human prostate (*6*). This inactivation was observed in homogenates prepared from freshly obtained human prostates as well as in the prostatic fluid obtained by massage of the prostate in normal individuals (*7*). Inactivation was never complete but stopped at the 40 to 50 percent level which remained constant thereafter until, on removal of calcium ions by the addition of sodium phosphate or Versene, restoration of the original level of enzyme took place (*8*).

We do not yet know the nature and mechanism of the interaction between calcium and acid phosphatase, but the following speculation may be of interest: There is a high concentration of citric acid in prostatic fluid (480 to 2700 mg/100 ml) and having now observed the effect of calcium on acid phosphatase, we think that this calcium-binding agent is able to prevent *in vivo* inactivation of the enzyme by the calcium ions normally present in the extracellular fluid of the prostate. Without this binding by citric acid, there might possibly occur an important loss of acid phosphatase in prostatic secretion through the mechanism described here. As far as the ionized calcium in blood serum is concerned, it is not high enough to cause rapid phosphatase inactivation; thus the elevated serum levels of acid phosphatase found in certain patients with carcinoma of the prostate cannot be increased by incubation of the serum with Versene as would be the case if inactivation by calcium took place in serum.

If the spermatozoa were devoid of acid phosphatase, one may speculate that the prostatic fluid could be a source of supply of this important enzyme, protected by citric acid, for the spermatozoa. All these considerations are hypothetical, but have the advantage of a provisional synthesis of available data, which should be abandoned as soon as new data will make it invalid.

The following facts characterize the action of calcium on acid phosphatase:

Inactivation by calcium is optimal at *p*H 7.5 and 37° C. In the lower *p*H range, the calcium effect is still present down to *p*H 3. At *p*H 3 and lower, there is a nonspecific, irreversible destruction of acid phosphatase which has nothing to do with calcium. The same is true at *p*H 7.8 and higher.

The effect of temperature is interesting: The optimal temperature for calcium inactivation is 37° C; at this temperature maximum inactivation is usually reached within 20 to 30 minutes. At 30° C and lower, no inactivation takes place in 2 hours.

The calcium effect is completely reversible. Restoration of the original acid phosphatase level by incubation in the absence of calcium occurs in nearly the same time as does inactivation in the presence of calcium. Complete restoration is still possible after incubation of the partially inactivated acid phosphatase for as long as 6 hours at 37° C (*8*).

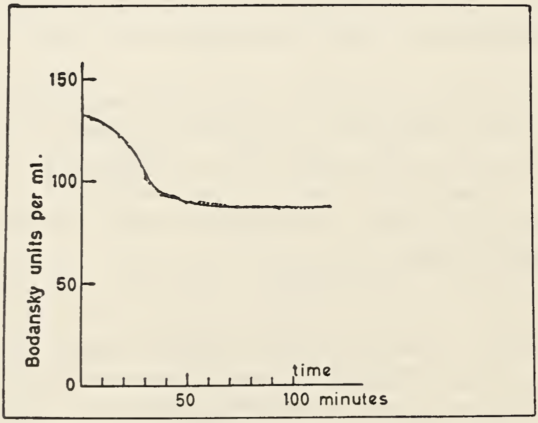
As to the amount of calcium necessary to promote inactivation, a concentration of 10 mg of $CaCl_2$ per 100 ml appears optimal, and the reaction is not modified in any way if this is increased to 40 mg per 100 ml.

It is possible to realize a marked degree of purification of the prostatic acid phosphatase by ammonium sulfate fractionation. This purified preparation reacts with calcium exactly as with the nonpurified enzyme.

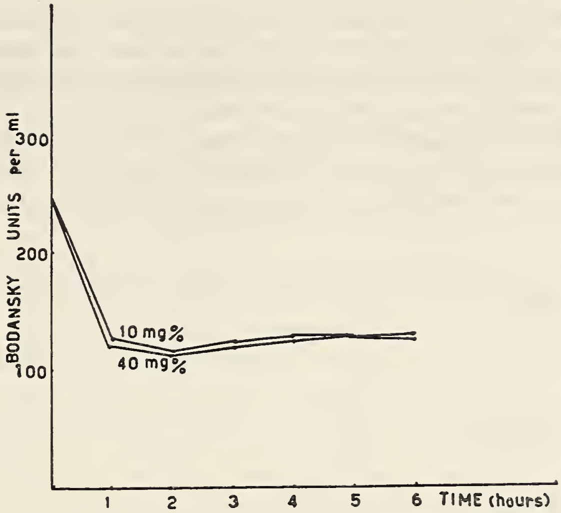
Of interest in connection with experiments to be described later is the observation that the addition of blood serum to prostate homogenates does not in any way modify the calcium effect on acid phosphatase, provided that the optimum *p*H range of the reaction is maintained, which requires special precautions when blood serum is used (*8*).

We were intrigued from the beginning by the fact that inactivation of acid phosphatase by calcium ions was never complete, but usually stopped at the level of approximately 50 percent of the original activity. The shape of the curve of inactivation, when determined at 2-minute intervals, is smooth and reaches a maximum between 20 and 30 minutes, leveling off gradually and remaining horizontal thereafter. The shape of the curve is not modified by an increase in the amount of calcium (text-figs. 1 and 2).

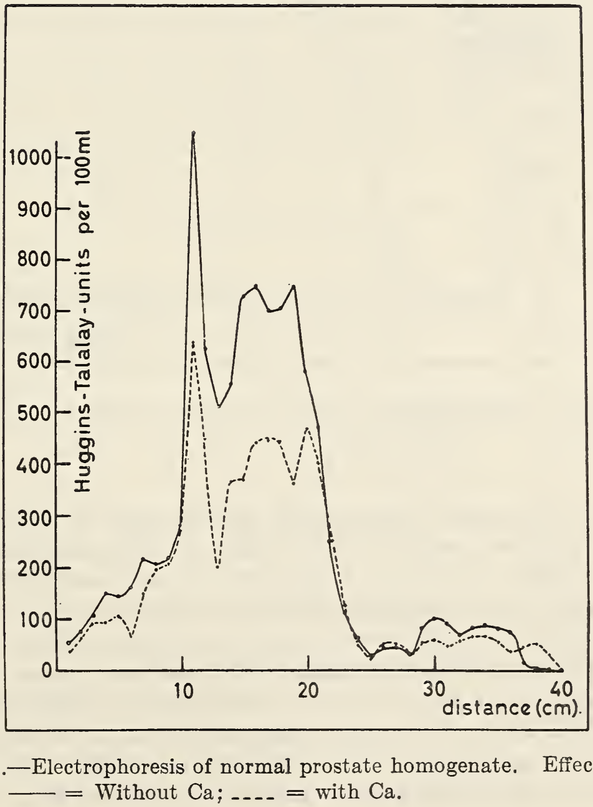
Additional information was also obtained from electrophoresis experiments on purified prostate acid phosphatase and homogenates, with the



Text-figure 1.—Effect of calcium (20 mg %) on acid phosphatase (prostate homogenate), *p*H 7, 37° C.

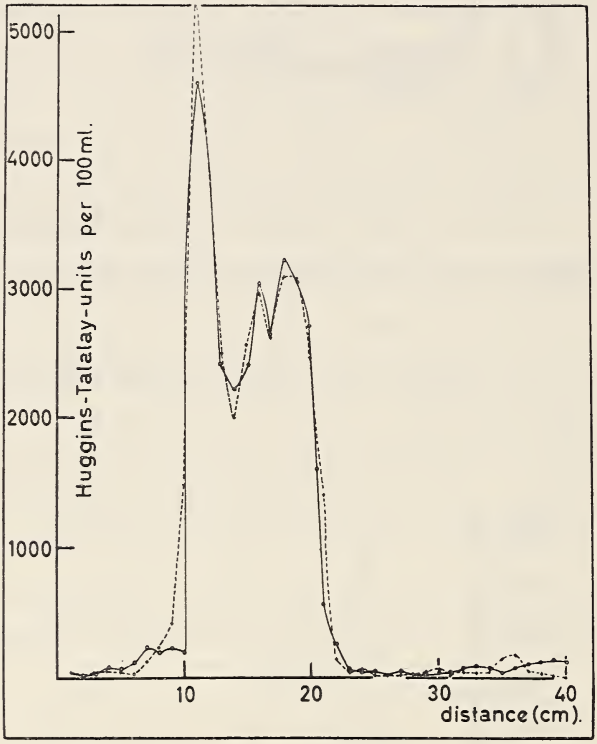


Text-figure 2.—Effect of different concentrations of Ca^{++}.



Text-figure 3.—Electrophoresis of normal prostate homogenate. Effect of calcium (20 mg %). —— = Without Ca; ---- = with Ca.

method described by Davidson (*9*). It was easy to demonstrate migration of the enzyme as reported by this author. Electrophoresis was repeated after the enzyme preparation had been incubated with calcium ions. Inactivation was clearly demonstrated on the electrophoretic curves, and these also showed that inactivation affected all the fractions of acid phosphatase with the same intensity, instead of suppressing completely one or two fractions and ignoring the others (text-figs. 3 and 4).



TEXT-FIGURE 4.—Electrophoresis of cancer prostate homogenate. Effect of calcium (20 mg %). ---- = Without Ca; —— = with Ca.

Therefore the partial character of calcium inactivation cannot be said to reflect different sensitivities of different acid phosphatase fractions. It could be the result of an equilibrium between two hypothetical forms of the enzyme, one combined with calcium and the other free. This will have to be tested by future work and it seems that there is justification for attempts to elucidate the nature of this reaction because of its possible significance in medicine, as shown by the following considerations. These concern the study on the calcium effect on patients with carcinoma of the prostate and metastases. We had been wondering, for a long time, whether the acid phosphatase produced by the carcinoma is really the same as the normal acid phosphatase. They seemed to be the same by the usual criteria, but now a new property of the phosphatase was available for tests of their similarity. It was not unreasonable to consider the possibility of small alterations developing in the chemical structure of an enzyme during carcinogenesis, which would be evidenced by a modification of the calcium effect. Therefore we studied calcium inactivation in cancer of the prostate. There are difficulties in securing satisfactory samples of prostatic carcinoma tissue. These samples should be obtained from metastatic lesions and not from the primary tumor to avoid all possible admixture of cancer tissue with normal prostatic tissue. Patients with easily removable metastatic lesions are few. However, some experiments on homogenates of pure prostatic carcinoma tissue have been carried out. Inactivation experiments have also been conducted on the acid phosphatase present in the serum of patients with carcinoma of the prostate and metastases. In the latter instance, there is every reason to believe that the acid phosphatase originates in the carcinoma. But we knew that the presence of serum did not modify the sensitivity of the normal acid phosphatase to calcium. Therefore experiments on the acid phosphatase present in the serum of patients with carcinoma are probably as valid as experiments carried out on homogenates from the carcinoma themselves.

Ten experiments were carried out on 5 patients from whom metastatic prostatic tissue could be obtained. Of these, 2 had never received hormonal treatment, 1 had received a single dose of diethylstilbestrol *per os* 4 days before the experiment, and 2 were being treated by estrogens more or less continuously. Of these 5 patients, 3 had elevated levels of serum acid phosphatase (table 1).

Our observations show that inactivation by calcium did *not* take place in homogenates from carcinoma of the prostate. Likewise, the serum acid phosphatase did not decrease when incubated with calcium ions. We have control experiments showing that increasing the amount of calcium ions to 40 mg did not produce inactivation of the acid phosphatase from carcinoma to any greater degree than did the usual concentration of 10 mg per 100 ml.

No explanation is yet available for this different response to calcium between the normal and the cancer acid phosphatases. It is possible that a chemical dissimilarity exists between the two enzymes which is responsible for the different reactivity to calcium ions, though other characteristics and properties remain the same in both. This dissimilarity could be associated with the carcinomatous transformation of the tissue, but this is just a speculation and there is not the beginning of demonstration of this possibility. In any event, calcium inactivation of acid phosphatase, which we first believed to be a purely biological curiosity, seems now to be acquiring some clinical significance.

In conclusion, a study of two enzymes found in prostatic tissue has been presented. Fibrinolysin is interesting because it produces a specific hemorrhagic disease when it is released in the blood stream. This occurs

Table 1.—Cancer of prostate: action of calcium on acid phosphatase

Experiment	Patient	Source of material	Initial value†	Values after incubation*			
				I Ca^{++} free (Versene), 90 minutes	II With Ca^{++}‡	III Reactivation (Versene) §	IV Ca^{++} free (Versene), 150 minutes
1	D.H.	Metastasis	100 (422)	114	99	104	130
2	D.H.	Metastasis	100 (382)	94	91	99	92
3	D.H.	Metastasis	100 (380)	95	94	94	94
4	LER.	Metastasis	100 (917)	100	95	104	101
5	LER.	Metastasis	100 (960)	99	90	97	99
6	RON.	Metastasis	100 (65)	106	98	98	95
7	RON.	Metastasis	100 (23)	101	91	91	87
8	LEG.	Metastasis	100 (58)	101	98	96	96
9	NIC.	Metastasis	100 (188)	147	98	159	147
10	NIC.	Metastasis	100 (156)	142	96	125	142
11	D.H.	Serum	100 (73)	99	96	96	96
12	BRU.	Serum	100 (25)	94	93	96	90
13	KAY.	Serum	100 (11)	100	95	98	100

*Ninety minutes at 37° C; values expressed in percentage of initial value.

†Numbers in *parentheses* are the actual values in Bodansky units per ml.

‡Sixty minutes at 37° C after inactivation.

§$CaCl_2$: 10 mg per 100 ml.

in certain patients with advanced carcinoma of the prostate. Acid phosphatase appears to be inactivated by calcium ions when it originates in the normal prostate, but not when it originates in a prostatic carcinoma. Both enzymes appear to be "hormone-dependent" under certain circumstances.

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DISCUSSION

Dr. Brendler: First question: Does calcium lower the serum acid phosphatase in normal persons? Second question: Are there any other inhibitors of prostatic acid phosphatase besides the calcium ion?

Dr. Tagnon: We have not studied the effect of calcium ions on the serum acid phosphatase of normal persons. The level is low and our impression was that fluctuations of this level, under experimental conditions, might not be significant because of this initial low level. Regarding the second question, there are other inhibitors but we have not studied them.

CITRIC ACID IN METASTASIZING CARCINOMA OF THE PROSTATE GLAND[1]

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You have heard how we were able, by utilizing the excellent grafting technique of Dr. Dorothy Price, to demonstrate that grafts of rat prostatic tissue would grow and produce the characteristic "marker metabolite," namely citric acid, in complete anatomical separation from the male reproductive tract, provided that the graft-bearing host animal remained under androgenic control (*1*). From the work of Huggins (*2*) and others there is evidence that the growth of human prostatic cancer, and its metastases, is also stimulated by androgenic steroids. We wanted to find out what the level of citric acid is in primary tumors of the prostate, and whether the ability to produce citric acid is also preserved in disseminated and cancerously altered prostatic tissue of the metastases. We obtained suitable human material at the Urological Clinic, University of Innsbruck, Innsbruck, Austria; all samples were examined histologically to obtain evidence of the type and extent of cancerous changes. We found (*3*) that values for citric acid in primary prostatic tumors were lower, 30 to 40 mg per 100 g, than those in normal human prostate, 800 to 2000 mg per 100 g. In para-aortal lymph nodes consisting almost entirely of solid undifferentiated cancerous tissue, the citric acid content was 46 mg per 100 g versus 0.8 to 1.6 mg per 100 g in normal lymph nodes. In one instance it was possible to determine separately citric acid in prostatic metastases in the liver and in surrounding pathologically unchanged liver tissue; the values per 100 g were 28.4 mg and 2.3 mg of citric acid, respectively, for cancerous and normal liver tissue.

It appears therefore that the ability to produce citric acid resides to a large extent not only in the primary tumor of the prostate, but also is still preserved (most interestingly) in metastasizing cancer located away from the male reproductive tract itself. In the course of fairly extensive operative procedures (transurethral resection of prostate) a relatively large amount of citric acid was found in a concentrate made from the bladder irrigation fluid. There was also an indication that surgical interference with the delicate prostatic tissue caused a transient small, but distinct, rise in the level of citric acid in peripheral blood.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

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METABOLISM IN VIVO OF Δ^4-ANDROSTENE-3,17-DIONE-7-H^3 AND ITS LOCALIZATION IN THE RAT[1]

WILLIAM H. PEARLMAN,[2] Department of Biological Chemistry and the Department of Surgery, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Massachusetts

The results of our experiments on the metabolism *in vivo* of Δ^4-androstene-3,17-dione-7-H^3 and its localization in the ventral prostate and other tissues of the rat have been published in detail (*1*). I am grateful for this opportunity to discuss these experiments and the concepts that led us to the study and to related studies on the metabolism and localization of tritium-labeled estrogen and progesterone in women with breast cancer (*2*) and in normal, intact female rats (*3*).

In constructing theories regarding the biochemical mechanisms underlying the biological action of steroid sex hormones—for example, the action of androstenedione or testosterone on a target sexual tissue such as the ventral prostate—it might be helpful to know: (*a*) the precise nature and molecular concentration of androgen to which the ventral prostate is exposed via the systemic blood plasma under normal physiological conditions, and (*b*) the precise nature and molecular concentration of androgen within the target organ itself. As the concentration of endogenous androgen in tissues is much too low to be detected by conventional methods, we have carried out metabolism studies *in vivo* with steroid sex hormones of unusually high specific activity. Isotopic labeling of various steroid hormones was achieved (*4*) in our laboratory by catalytic reduction with gaseous tritium of a double bond at position 6(7) in an appropriate steroid substrate (text-fig. 1). In steroids possessing a $\Delta^{4,6}$-diene-3-one structure, the double bond at position 6(7) is more reactive than that at 4(5) and undergoes catalytic reduction first. Tritium is thereby introduced at positions C–6 and C–7; tritium at position C–6 is labile and can be removed with alkali. In this manner, Δ^4-androstene-3,17-dione-7-H^3 and also progesterone-7-H^3 were prepared (text-fig. 2). We have also reported (*5*) the preparation of estrogen labeled with tritium at positions C–6 and C–7 employing 6-dehydroestrone as substrate in the catalytic reduction procedure. In this manner, specific activities of the order of several curies per mM can be obtained; this permits the detection and estimation of 1/10,000 of a μg or less of the steroid.

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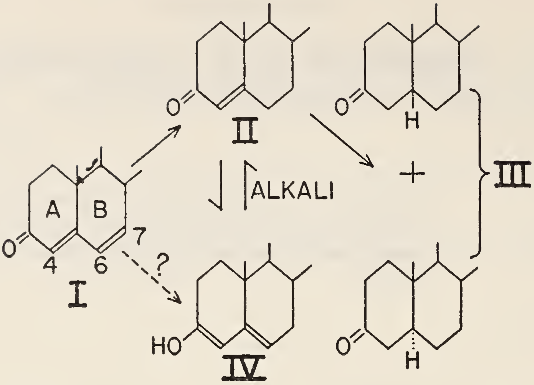
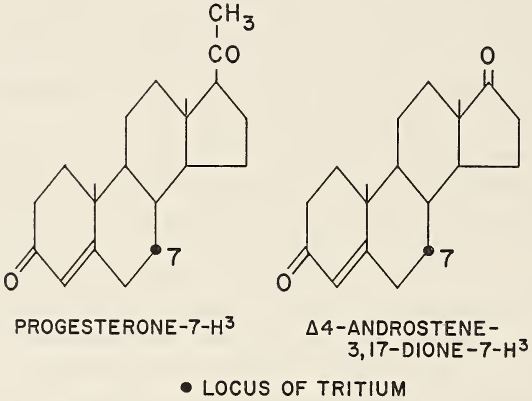
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] Recipient of a Research Career Development Award, U.S. Public Health Service.



TEXT-FIGURE 1.—The course of catalytic hydrogenation of $\Delta^{4,6}$-diene-3-ketonic steroids (I); only rings A and B are represented.



TEXT-FIGURE 2.—Tritium-labeled androstenedione and progesterone.

We next considered the mode of administering the radioactive hormone to the experimental subject. An attempt was made to simulate physiological conditions of endogenous hormone secretion by infusing Δ^4-androstene-3,17-dione-7-H^3 intravenously at a slow and constant rate over a long period in order to approach a steady state. It seemed more advantageous to determine radioactivity concentration in the various tissues at dynamic equilibrium than to follow the rapid and variable rates of disappearance of the hormonal radiometabolites, observed in experiments in which the radioactive hormone is injected in a single rapid dose.

RESULTS

Two experiments were carried out on intact adult male rats. In experiment 1, 3.38 mc of Δ^4-androstene-3,17-dione-7-H^3, specific activity

3.82 mc per mg was infused in 200 minutes and, in experiment 2, 2.31 mc in 140 minutes. Table 1 gives the concentration of radioactivity in the various tissues at the end of the infusion period. When millicuries of hormone were intravenously administered, only microcuries were found in the systemic plasma and in other tissues. Also the radioconcentration in the ventral prostate is distinctly higher than that in plasma or skeletal muscle. The presence of androgen radiometabolites in muscle is interesting, because this tissue may be regarded as a target organ, though a nonsexual one, in view of the stimulatory effect of androgen on protein metabolism; this radioactive material, however, was not investigated further. The radioconcentration in the liver is very high, at least 6 times higher than the plasma radioconcentration. From the tissue radioconcentration and the specific activity of the androstenedione administered, the concentration of total hormonal radiometabolites in the plasma and ventral prostate may be estimated to be of the order of 10^{-6}M. Inasmuch as the rate of infusion of radioactive androstenedione probably exceeded that of endogenuous androgen, it seems likely that the concentration of endogenous androgen in the ventral prostate may be as low as 10^{-7}M.

TABLE 1.—Concentration of radioactivity in rat tissues

Experiment No.	Tissue extracted	Amount of wet tissue extracted	Acetone extract		Methanol extract		Concentration of extractable radioactivity in tissue
			Radioactivity	Weight	Radioactivity	Weight	
		ml or g	*μc*	*mg*	*μc*	*mg*	*μc/ml or g*
1	Plasma	4.0	1.91	12.2	$<$0.01	11.2	0.48
	Ventral prostate	0.520	0.371	12.2	0.015	11.4	0.74
2	Plasma	3.5*	1.43	34.9	0.026	6.5	0.416
	Blood cells	*	0.59	37.2	0.035	14.0	
	Ventral prostate	0.386	0.25	9.5	0.001	9.6	0.65
	Muscle†	0.771	0.23	14.1	0.001	15.2	0.30
	Liver	6.90	21.1	292	1.77	138	3.32

*The plasma and the red blood cells were derived from 7.1 ml of whole blood.
†Thigh muscle from the noninjected leg was removed for the analysis.

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Table 2 shows that the turnover time of the hormonal radiometabolites in systemic plasma is extremely short. The turnover time is the ratio of plasma radioactivity in the total circulation to the rate of infusion under conditions of steady state.

The foregoing data merely indicate radioconcentration. But what is the precise nature of this radioactive material? Our knowledge of the metabolism of androstenedione (or testosterone), particularly with respect to its biological inactivation, is summarized in text-figure 3. It may be recalled that the metabolites in the 5α-androstane series, *e.g.*, androsterone, are androgenic, whereas those in the 5β-androstane series, *e.g.*, etiocholanolone, are devoid of such activity. The conjugated metabolites

Table 2.—Turnover time of radiometabolites of Δ^4-androstene-3,17-dione-7-H^3 in systemic plasma

Turnover time under conditions of steady state was calculated from the equation, $T = CV/r$, where T is the turnover time, C the concentration of radioactivity in systemic plasma, V the total volume of circulating plasma estimated from the body weight, and r the constant rate of intravenous infusion of Δ4-androstene-3,17-dione-7-H^3. Plasma radioactivity comprises Δ4-androstene-3,17-dione-7-H^3 and its metabolites in the plasma extract.

Experiment No.	C	V	r	T
	μc/ml	*ml*	*μc/min*	*min*
1	0. 48	7. 45	16. 9	0. 21
2	0. 416	6. 8	16. 5	0. 171

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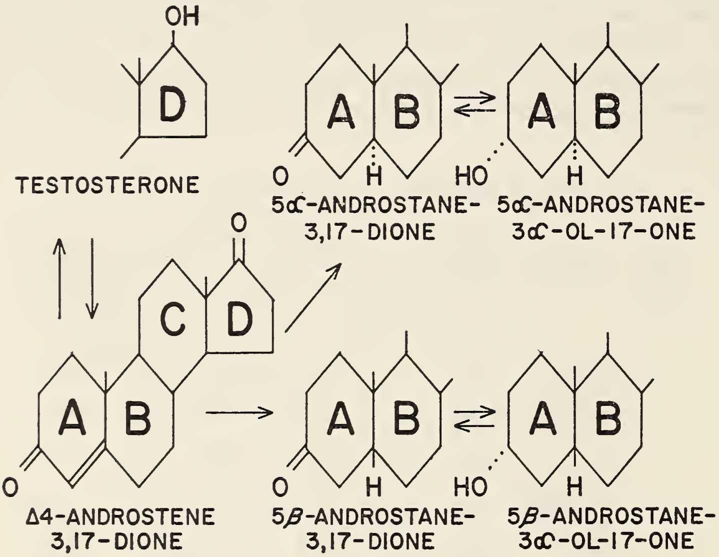

Text-figure 3.—Major pathways in the metabolism of androstenedione.

are very weakly androgenic or perhaps lacking altogether in biological activity. Thus, one may expect the androgenic activity of the plasma to reside in the free (ether-soluble) fraction. Our primary interest is in the androgenically active metabolites because these determine the degree of response elicited in target sexual tissues. With the preceding metabolic scheme in mind, the tissue extracts were partitioned into the free (or ether-soluble) and conjugated (or water-soluble, butanol-soluble) fractions.

Table 3 gives the ratio of free to conjugated radioactivity in extracts of systemic plasma, ventral prostate, and liver. This ratio is highest in the ventral prostate and lowest in the liver.

Table 4 summarizes the results obtained on partition column chromatography of the free radioactive material in extracts of the ventral prostate and plasma; isotope dilution analysis of the appropriate eluate fractions

TABLE 3.—Partitioning of certain tissue extracts into free and conjugated radiometabolite fractions

Experiment No.	Tissue extracted	Radioactivity in		Ratio of free to conjugated radioactivity
		Ether-soluble, 90% methanol fraction	Water-soluble, butanol-soluble fraction	
		μc		
1	Plasma	1. 32	0. 57	2. 32
	Ventral prostate	0. 288	0. 046	6. 25
2	Liver	8. 60	8. 72	0. 99

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was performed. It is evident that there are present in the ventral prostate a variety of hormonal radiometabolites, most of which possess androgenic activity. The ratio of androsterone (in the 5α-androstane series) to etiocholanolone (in the 5β-androstane series) is high; in other words, the accumulation of the biologically active stereoisomer is favored. The pattern of free hormonal radiometabolites in the plasma does not appear to differ very much from that of the ventral prostate. Apparently the concentration and composition of androgenic products in the ventral prostate reflect those found in the systemic plasma under conditions of steady state. The radioactive hormone, androstenedione, which was originally administered, constitutes only a small fraction of the total plasma radioactivity.

Table 5 indicates that the pattern of free hormonal radiometabolites in the liver is strikingly different from that seen in either the plasma or

TABLE 4.—Identification and estimation of free radiometabolites in plasma and ventral prostate extracts

Pooled eluate fractions		Total radioactivity chromatographed		Steroid identified	Radioactivity in respective pooled eluate fractions attributable by isotope dilution analysis to steroid	
Plasma	Ventral prostate	Plasma	Ventral prostate		Plasma	Ventral prostate
		%			%	
3–6	3–5	34	14	Testosterone	90	47
9	6–9	31	23	5α-Androstane-3α-ol-17-one	14	58
				5β-Androstane-3α-ol-17-one	1. 2	3. 5
11–17	10–15	19	5. 8	Δ4-Androstene-3,17-dione	111	96
(19)	18–29		21	5α-Androstane-3,17-dione		71

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ventral prostate. Androstenedione or testosterone is virtually absent; and unidentified highly polar radiometabolites (in eluate fractions 3–5) are present in very high proportion. The ratio of androsterone to etiocholanolone is very low, which is quite the reverse of what is found in the ventral prostate or plasma, *i.e.*, the accumulation of the androgenically inactive stereoisomer is favored in the liver.

Table 5.—Free radiometabolites in liver extracts

Pooled eluate fraction	Total radioactivity chromatographed	Steroid identified	Radioactivity in respective pooled eluate fractions attributable by isotope dilution analysis to steroid
	%		%
3–5	55.0	Testosterone	<1.2
6–9	18.1	5α-Androstane-3α-ol-17-one	<4.1
		5β-Androstane-3α-ol-17-one	25.6
11–16	~2	(Δ4-Androstene-3,17-dione)	
19–27	~3	(5α- and 5β-Androstane-3,17-dione)	
"Stripping"	~6		

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SUMMARY

One may conclude that the androgenic hormone administered in these infusion experiments was rapidly metabolized and removed from the circulation. The concentration of radioactivity in the liver was much higher than that in plasma, and the pattern of radiometabolites in liver differed widely from that in the circulation. It appears that the liver in the rat plays an important role in metabolizing androgen, conjugating and eliminating it into the bile.

The free or unconjugated radioactive products that were isolated and quantitatively estimated from the plasma and the ventral prostate included testosterone, androsterone, etiocholanolone, Δ^4-androstene-3,17-dione, and 5α-androstane-3,17-dione. The pattern of radiometabolites in the ventral prostate resembled that in the plasma, but the concentration of radioactivity in that sexual organ was significantly higher than that in plasma or muscle, suggestive of a selective process in operation in the prostate. The concentration of steroidal radiometabolites in systemic plasma and in the ventral prostate was calculated to be of the order of 10^{-6}M; the concentration of endogenous androgen in the ventral prostate may be considerably less.

The free radiometabolites in liver were predominantly highly polar and were not identified; of the less polar radiometabolites present, etio-

cholanolone was isolated and quantitatively estimated. The nature of the conjugated radiometabolites in the liver and other tissues was not explored; the ratio of conjugated to free radioactivity was highest in the liver.

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COMMENTS ON DR. PEARLMAN'S PAPER; COMPARISON OF ANDROGENS AND ESTROGENS AS TO THEIR FATE IN TARGET TISSUES [1, 2]

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In considering the ability of gonadal hormones to promote growth and maintain function in the accessory reproductive tissues, it is often convenient to regard the stimulation of the prostate and seminal vesicles by androgen in the male as being somewhat analogous to the stimulation of the uterus and vagina by estrogen in the female. In fact, there is a temptation to consider a unified theory for the mechanism of sex hormone action. But in spite of many aspects of similarity, all of us realize that there are certain important differences between the hormonal actions of the androgens and those of the estrogens. The estrogens are active in considerably lower doses than are the androgens, and cellular responses to them are evident much more rapidly. Although you heard earlier of a nonsteroid which showed androgenic activity, still we must admit that there are no simple, nonsteroidal androgens comparable to diethylstilbestrol and hexestrol exhibiting full estrogenic activity at the same dose level as the natural hormones. I shall use the interesting and elegant experiments just reported by Dr. Pearlman, as well as some of our own results, to point out two further differences between estrogens and androgens concerned with their incorporation and fate in the growth-responsive tissues.

You have seen from Dr. Pearlman's presentation that, after the administration of androstenedione, the rat prostate contains a mixture of steroidal substances. Whether the metabolic transformations leading to these products actually take place in the prostate and are related to growth process is difficult to say, but at least the compounds are there. Moreover, Harding and Samuels (*1*) have shown that, after the administration of testosterone, androstenedione appears in the prostate. Similar observations in Dr. Mann's laboratory were mentioned earlier. However, we have shown (*2*) that when one administers physiological amounts of estradiol to the rat, the uterus and vagina contain only free estradiol. Recently we obtained analogous results with 17α-methylestradiol and hexestrol. Using mixtures of 6,7- and 17-tritiated estradiol, we have estab-

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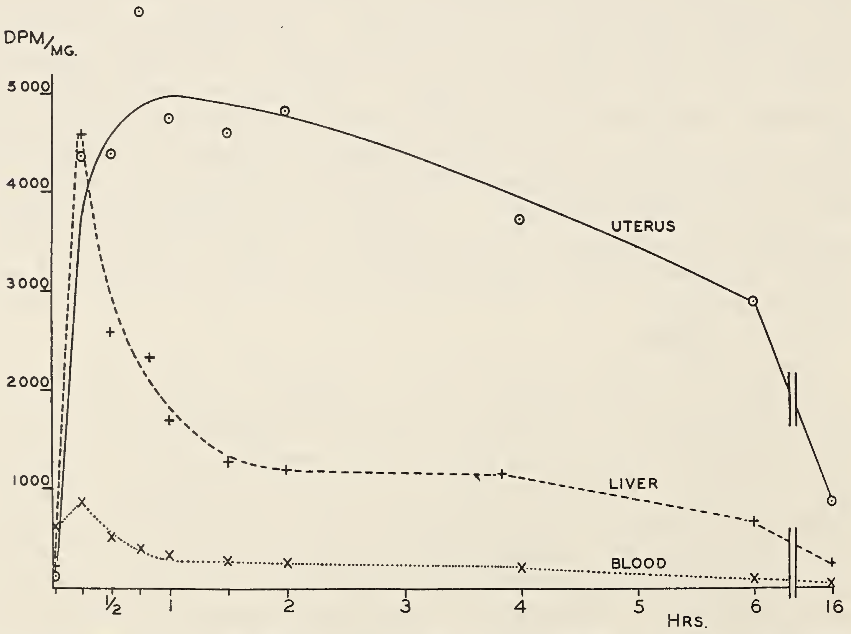
[1] Presented at the Workshop in Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] Supported in part by contract No. SA-43-ph-4317, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

lished, at least to our own satisfaction, that the rat uterus *in vivo* does not have the capacity to oxidize estradiol to estrone. It would appear that estradiol, 17-methylestradiol, and hexestrol all stimulate growth of the uterus and vagina without undergoing metabolic change, whereas androgen action may involve chemical transformation of the steroid molecule.

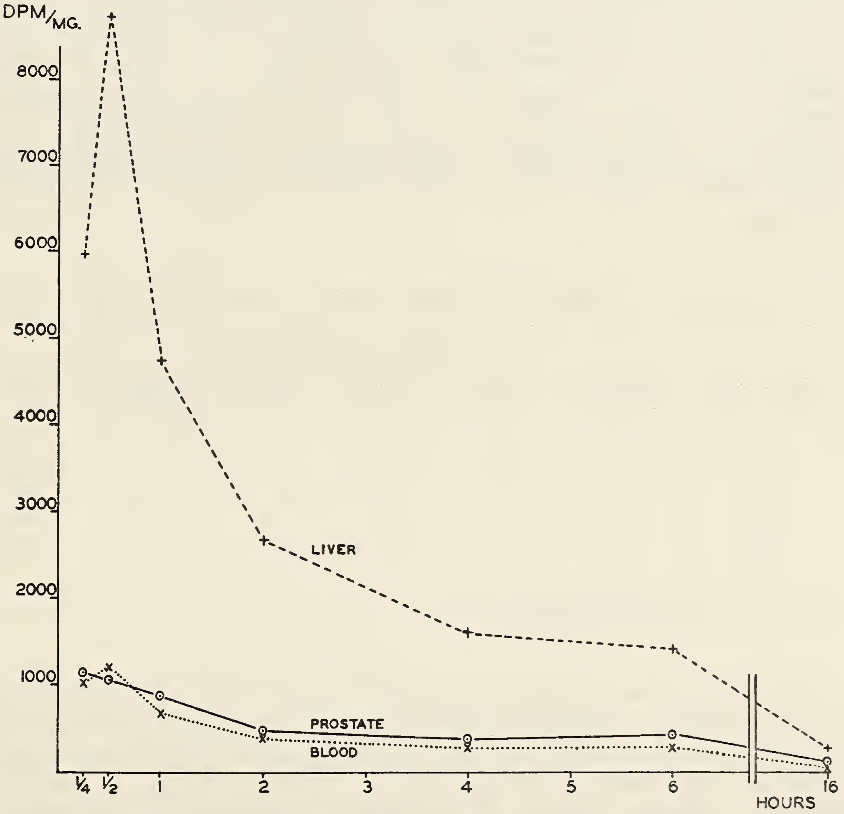
A further difference between estrogens and androgens is evident from their comparative patterns of incorporation into target tissues. We have reported (*2*) that the uterus and vagina of the immature Sprague-Dawley rat show a characteristic ability to incorporate and retain estradiol (also 17α-methylestradiol and hexestrol but not estrone) in contrast to various other tissues, such as liver, kidney, muscle, bone, and adrenal, in which the steroid level parallels that of the blood. This affinity of uterus and vagina for the hormone is manifest when the steroid is administered either in saline from which absorption is rapid or in oil from which absorption is gradual. For the purpose of comparison with the incorporation pattern of androgen into the prostate, an analogous experiment was performed with the "prestimulated" immature rats. These animals received 0.1 μg of nonradioactive estradiol in saline when 22 and 23 days of age, which caused the uterus to triple in size by day 24 when 0.1 μg of



TEXT-FIGURE 1.—Concentration of radioactivity in tissues of 24-day-old rat after single subcutaneous injection of 0.10 μg (11.8 μc) of estradiol-6,7-H^3 in 0.5 ml saline. Animals were prestimulated with 0.1 μg of nonradioactive estradiol on days 22 and 23. Liver points are means of 4 aliquots of dried pooled tissue from 6 animals; other points are median values of 6 individual animals. Blood data plotted as DPM/5 μl; other data as DPM/mg dry tissue.

radioactive estradiol in saline was administered. The characteristic ability of the uterus to incorporate and retain radioactive estradiol is clearly evident (text-fig. 1).

For a study of the incorporation pattern of testosterone into rat tissues, Sprague-Dawley animals were given 500 μg of nonradioactive testosterone propionate in sesame oil on days 22 and 24 of age and 100 μg on day 26 to develop the immature prostates and seminal vesicles to such a degree that the various lobes could be distinguished readily. Preliminary studies with radioactive hormone had demonstrated that with these doses practically no steroid is left in the tissues by day 28. To the prestimulated animals, 10 μg of 1,2-tritiated testosterone in saline was administered subcutaneously on day 28, and the tissues were analyzed for radioactivity at various intervals thereafter. The results in text-figure 2 show that the



TEXT-FIGURE 2.—Concentration of radioactivity in tissues of 28-day-old rat after single subcutaneous injection of 10 μg (27 μc) of testosterone-1,2-H^3 in 0.5 ml saline. Animals were prestimulated with 0.5 mg of nonradioactive testosterone propionate in sesame oil on days 22 and 24 and with 0.1 mg on day 26. Liver points are means of 4 aliquots of pooled dried tissue from 4 animals; other points are median values of 4 individual animals. Blood data plotted as DPM/5 μl; other data as DPM/mg dry tissue.

incorporation of radioactivity into liver and blood is not greatly different in pattern from that observed with estradiol. Radioactivity reaches maximum in 30 minutes and then decreases steadily. As was reported by Dr. Pearlman, the level of radioactivity in liver is considerably higher than in the other tissues examined. In contrast to the marked retention of estradiol by uterus and vagina in the female rat, the level of radioactivity in the ventral prostate, as well as in the seminal vesicles and coagulating gland, is practically the same as that in blood, with no indication of any special affinity of these tissues for the hormone.[3]

Similar conclusions can be drawn from an experiment in dogs which we carried out in cooperation with Dr. William W. Scott. A subcutaneous injection of 1,2-tritiated testosterone in saline was given and the radioactivity in several tissues determined at various intervals. From the results listed in table 1, it appears that the level of radioactivity in the blood shows a pattern similar to that in the rat, that is, highest at one-half hour and decreasing steadily thereafter. Radioactivity in the prostate tends to run a little higher than that in the blood and the other tissues examined, but there is no evidence of the prolonged retention of radioactive steroid comparable to that observed with estradiol in the uterus. It can be seen that a large amount of radioactivity is present in the urine, especially between 1 and 2 hours after administration of the hormone.

TABLE 1.—Radioactivity in dog tissues after testosterone-1,2-H^3*

	DPM per mg wet tissue					
Time (hrs)	Prostate	Blood	Muscle	Tongue	Bladder	Urine
0. 5	38	29	—	—	—	—
1. 0	—	14	—	—	—	2, 140
1. 5	—	12	—	—	—	2, 370
2. 0	10	—	7	8	9	740
2. 5	—	5	—	—	—	610
3. 0	6	4	—	4	4	—

*100 μg = 8.8 × 10^8 DPM, subcutaneously in saline.

In summary, these experiments in the rat and the dog, with the findings of Dr. Pearlman and others, indicate two differences between the way male tissues handle testosterone and the manner in which female tissues handle estradiol. The uterus and vagina show a striking affinity for estradiol, which the prostate and seminal vesicles do not show for testosterone. The female target tissues do not chemically alter the steroid molecule, whereas metabolic transformation products of the administered androgen are present in prostatic tissue.

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[3] Similar incorporation patterns for C^{14}-labeled testosterone were described recently by Harding and Samuels (*1*), though Butenandt, Günther, and Turba have reported that rat seminal vesicles take up considerably more radioactive testosterone than do liver and muscle (*3*).

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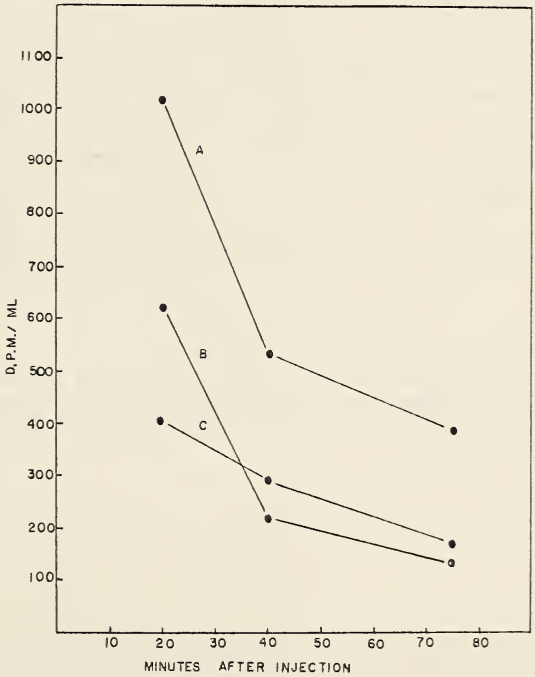
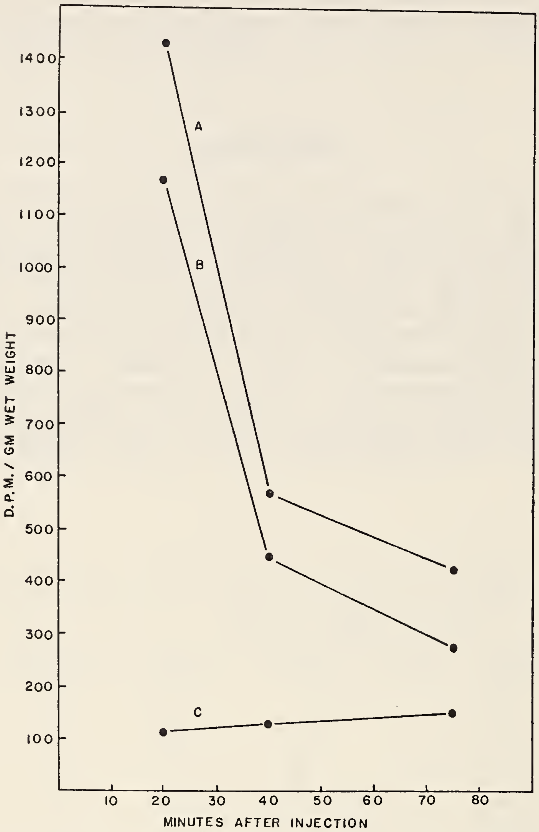
DISCUSSION

Dr. Harding: We have published results (Endocrinology 70: 109, 1962) differing from those that Dr. Jensen and Dr. Pearlman have presented. This difference is illustrated in text-figures 1 and 2. Text-figure 1 shows the radioactivity appearing in the blood of 250 to 300 g rats after the injection of 40 μg of testosterone-4-C^{14}. Line A is the total activity found by directly counting whole plasma, line B the chloroform-extractable radioactivity, and line C the 80 percent ethanol-soluble, chloroform-insoluble radioactivity, presumably steroid conjugates. The chloroform-soluble material is largely free testosterone. Text-figure 2 shows the radioactivity appearing in the ventral prostates of the same animals shown in text-figure 1. Line A is a direct count of the whole homogenates and lines B and C represent radioactivity



Text-figure 1.—Radioactivity in blood of 250 to 300 g rats after injection of 40 μg testosterone-4-C^{14}. Line A = total activity found by directly counting whole plasma. Line B = chloroform-extractable radioactivity. Line C = 80 percent ethanol-soluble, chloroform-insoluble radioactivity.

obtained by the same extraction procedure described for text-figure 1. Although the total radioactivity in the two tissues is approximately the same, it is apparent that the chloroform-soluble radioactivity of the ventral prostrate, which we have shown to be largely Δ^4-androstenedione, is twice that of the blood. One must conclude from our studies that there is a preferential uptake of free testosterone by the ventral prostate form the blood, and that in addition it is rapidly converted to Δ^4-androstenedione.



Text-figure 2.—Radioactivity in ventral prostates of same animals shown in text-figure 1. Line A = direct count of whole homogenates. Lines B and C = radioactivity by same extraction procedure described for text-figure 1.

TESTOSTERONE METABOLISM IN THE PROSTATE [1, 2]

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General Medical Research Laboratories, Veterans Administration Hospital, and Department of Biochemistry, State University of New York at Buffalo, Buffalo, New York

If one may assume that the trophic action of androgens on the prostate gland entails some active interplay between gland and hormone, it seems possible that the nature of this action might be indicated by the character of the resultant steroid products.

To obviate possible effects of serum proteins and known hepatic effects, we have studied metabolites of radiotestosterone obtained by incubation at *p*H 7.4 in Ringer's-phosphate buffer with minces of rat prostate (*1*) and both minces and slices of human benign hypertrophic glands obtained at suprapubic and transurethral section (*2*). Ether extracts of homogenized, incubated samples, which had been deproteinized with 80 percent alcohol, were chromatographed in various systems, and the products further characterized by mixed chromatography on paper and/or alumina columns and by recrystallization to constant specific activity.

The most abundant metabolites of testosterone, representing over 50 percent of the degradation products obtained both in the rat and human glands, were 5α-androstan-17β-ol-3-one, or dihydrotestosterone, and 5α-androstane-3α, 17β-diol (table 1), both of which are androgens of potency comparable to testosterone (15 μg/IU by the capon comb test). Smaller amounts of the less potent androgens, androstenedione and 5α-androstane-dione, probably represent intermediates, judged from our preliminary studies of metabolic routes. In addition to these principal products there was a significant amount of androsterone and a spectrum of what seemed to be 6-hydroxy derivatives of androstenedione and testosterone. More positive identification awaits procurement of authentic standards. The 6β-hydroxy androstenedione, which we have, checks perfectly with the most mobile of this group.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] This work was aided by grant C–2487 from the National Cancer Institute, National Institutes of Health, Public Health Service, and grant 62-VAH-4 from the United Health Foundation of Western New York.

[3] We wish to thank Dr. Andrew Gage for guidance in surgical procedure; Mr. Bohm in Dr. Arthur MacNiell's laboratory, Buffalo General Hospital, for help with the perfusion pump; Dr. Josef Fried, the Squibb Institute for Medical Research, Dr. Paul O'Connell, the Upjohn Co., and Dr. Allan H. Conney, Burroughs Wellcome & Co., for reference steroids; Dr. Pasquale Greco, the Millard Fillmore Hospital, and Dr. Maurice Gonder, of this hospital, for providing benign hypertrophic prostatic tissue; and Mrs. Carol Hughes, Mr. Arthur Cross, Jr., and Miss Micki Lawrence for expert technical assistance.

Table 1.—Metabolism of testosterone-1,2-H^3 by human benign hypertrophic prostate

Compound	Relative biological effect*		Yield (% total metabolites)
	Seminal vesicle (%)	Capon comb (%)	
5α-Androstan-17β-ol-3-one	200	75	35
5α-Androstane-3α,17β-diol	33	75	18
4-Androstene-3,17-dione	20	12	13. 8
Androsterone	10	10	8. 0
5α-Androstane-3,17-dione	14	12	7. 7
4-Androstene-6β-ol-3,17-dione	—	—	5. 3
Other more polar metabolites	—	—	12. 2

*Testosterone = 100 percent. Data from Dorfman (*3*).

Although these results agree with those of Goldzieher and Acevedo (*4*), they are at variance with the findings obtained on systemic administration of testosterone and androstenedione by Harding and Samuels (*5*) and Pearlman and Pearlman (*6*). It seemed probable that these differences were accountable either to the mode of presentation of steroid substrate to the gland or the nature of the substrate presented.

To compare the fate of the androgen administered to the gland via blood in the capillary bed with that found on *in vitro* presentation, the arterial supply to all structures in the pelvic region of anesthetized (Nembutal) rats, except the ventral prostate, was ligated, and the prostate perfused through the abdominal aorta and the vena cava with rat blood containing testosterone-1,2-H^3 (fig. 1). On chromatographic analysis of the perfusate and the perfused gland, the distribution of products was essentially identical in blood and gland (except for unmetabolized testosterone in the blood) and also identical to the products obtained *in vitro* (fig. 2). The androstenedione level of the perfused blood was relatively higher than that in the *in vitro* incubated samples, but, as seen in the scan of blood incubated with steroid in the absence of prostate tissue, this product can be produced by the blood alone.

In vivo, much of the testicular androgen would traverse the liver and undergo hepatic transformations before reaching the prostate. To see how the prostate acted on this modified substrate we did one experiment in which radiotestosterone was incubated *in vitro* with minced liver, and the extract, cleared of unmetabolized testosterone by chromatography, was presented to the minced prostate *in vitro*. Table 2 shows the percent distribution of liver metabolites, exclusive of a large quantity of highly polar radioactive material, to be principally androstenedione and dihydrotestosterone. No androsterone is present. The last column shows the prostatic metabolites of the products of the liver's action. There is still no androsterone and little change in the level of dihydrotestosterone. The prostate has metabolized the androstenedione completely to two products, X_1, more polar, and X_2, less polar, than the starting material. We have not yet identified these presumed ketosteroids.

It is obvious that the interpretation of prostatic steroidolytic activity is most complex in the intact organism. We believe that the close agree-

Table 2.—Prostatic metabolites of liver metabolites of testosterone

Compound	R_t*	Metabolites (%)	
		Liver	Prostate
5α-Androstan-17β-o1-3-one	3.1	59.2	67.2
Androsterone	3.8	2.0	4.2
X_1	4.91		16.5
4-Androstene-3,17-dione	5.9	38.8	0
X_2	6.6		11.4

*R_t testosterone = 1.0.

ment between the metabolites in the perfused gland and those in the minced gland adds credibility to our claims that these are the true products of prostatic activity. As such, they demonstrate the presence in the rat ventral prostate and in the human benign hypertrophic gland of the following enzymes: 5α-reductase, 17β-dehydrogenase, 3α-dehydrogenase, and 6β-hydroxylase. It is hoped that, through further study of the metabolic pathways, clues may be obtained as to the nature of steroid mediation of prostatic biosynthetic processes.

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Plate 33

Figure 1.—Perfusion of rat prostate. Blood passes from reservoir successively through pump, heat exchanger, aorta, prostate, and vena cava to the oxygenerator, from which it is returned intermittently to reservoir. Pumping rate is adjusted to maintain a pressure of 90 to 120 mm Hg. (A paper, by Farnsworth, Brown, and Lawrence, giving details of the operative setup, evolved by Miss Lawrence, and of the apparatus, designed and built by one of us, JRB, will be published in *Endocrinology*.)

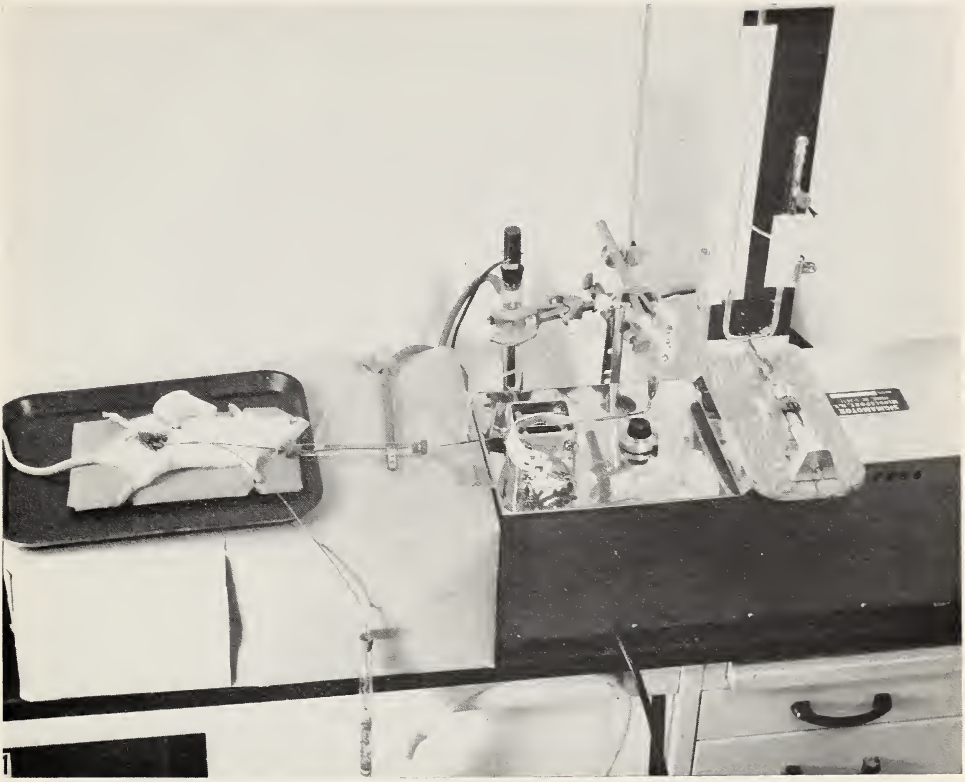
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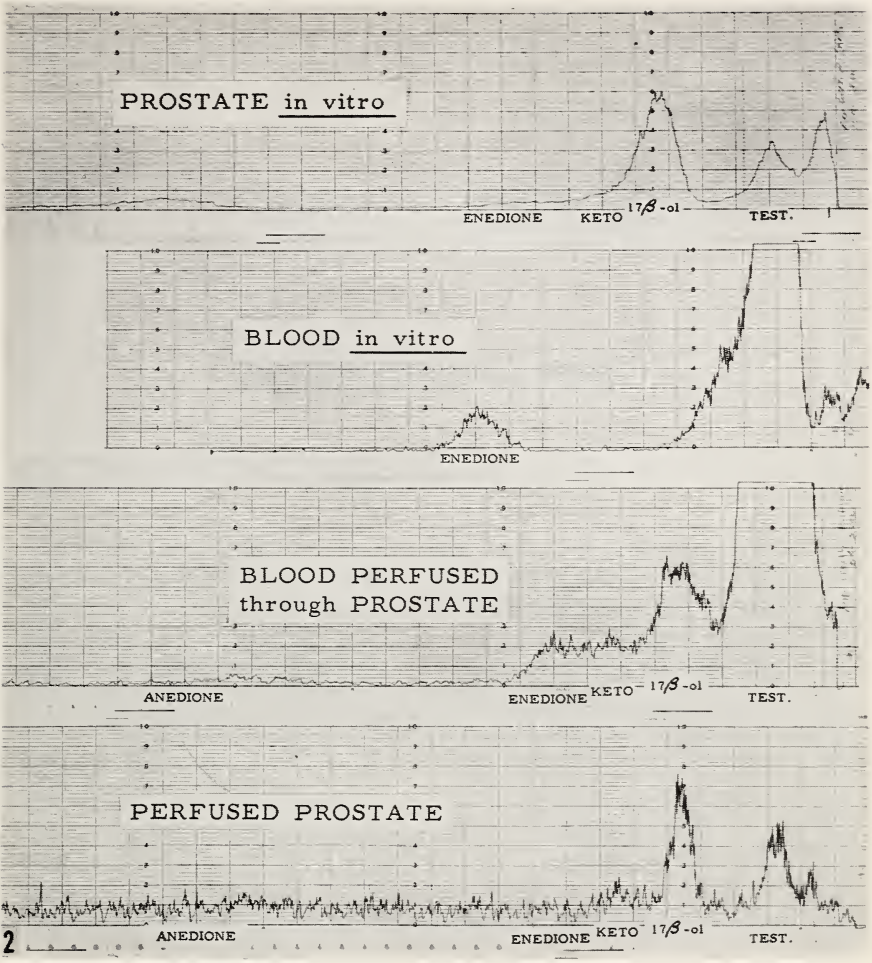
PLATE 34

FIGURE 2.—Metabolites of testosterone. Scans of distribution of radiosteroid on ligroin-propylene glycol-methanol chromatograms of extracts. Abbreviated names of steroids indicate location of reference compounds. Key: "anedione," 5α-androstane-3,17-dione; "enedione," 4-androstene-3,17-dione; "keto," ketosteroid (androsterone); "17β-ol," 5α-androstan-17β-ol-3-one; "test.," testosterone.


PROSTATE in vitro
ENEDIONE
KETO
17β-ol
TEST.
BLOOD in vitro
ENEDIONE
BLOOD PERFUSED through PROSTATE
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17β-ol
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KETO
17β-ol
TEST.

2

DISCUSSION ON BIOCHEMISTRY; FATTY ACID SYNTHESIS IN THE RAT VENTRAL PROSTATE[1]

NINA HOLLANDER, Department of Biochemistry, University of Virginia, Charlottesville, Virginia

There is reason to believe that fatty acid synthesis in the prostate is under hormonal control. Dr. Williams-Ashman showed decreased incorporation of C^{14}-acetate into fatty acids by the rat prostate after castration, which could be restored to normal by the administration of testosterone. This, however, does, not tell us the site of testosterone action. We were very interested in Dr. Farnsworth's report of an *in vitro* effect of testosterone on the incorporation of C^{14}-acetate into fatty acids by rat prostate mince.

We have done a number of experiments somewhat similar to those reported by Dr. Farnsworth and have had considerable difficulty with replication in this system. We found significant stimulation of fatty acid synthesis from C^{14}-acetate, C^{14}-pyruvate, and C^{14}-citrate by 10^{-7} M testosterone. With pyruvate and citrate as precursors, the effect can be demonstrated only in the presence of bicarbonate, in a system fortified with ATP, coenzyme A, and glucose. Unfortunately, we have obtained similar results by using cholesterol, progesterone, or estradiol instead of testosterone, at the level of 10^{-7} M in the *in vitro* system.

Because of the difficulties with whole tissue, we later turned to the study of partially purified enzymes concerned with fatty acid synthesis. As reported by Wakil, Vagelos, and others, acetyl coenzyme A carboxylase is the enzyme which limits the rate of fatty acid synthesis in liver and adipose tissue. The activity of this enzyme is stimulated by the presence of citrate and other citric acid cycle intermediates. We have found this enzyme in homogenates of rat ventral prostate. It is stimulated by as little as 10^{-4} M citrate. There is no striking change in its activity 1, 2, or 3 days after castration. Preliminary results suggest that the enzyme from castrate tissue may have increased sensitivity to citrate. This and related aspects of fatty acid synthesis in prostate are being investigated further.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962

DISCUSSION ON BIOCHEMISTRY; OBSERVATIONS ON THE CANINE AND RAT PROSTATE [1, 2]

HARRIS ROSENKRANTZ, Mason Research Institute, Worcester, Massachusetts

Many investigators have presented the results of their studies on the prostate gland, elucidating its physiology and biochemistry. Some of their findings predicted the usefulness of bioassay procedures utilizing weight changes in the rat ventral prostate and volume variations in regard to canine prostatic secretion as a means of estimating hormone potency.

In a series of explorations in our laboratory, both assay procedures were further examined. Analysis of the biochemical content of canine prostatic fluid indicated the following distribution of materials: 25 percent ash, 2.4 percent lipides, 6.9 percent carbohydrate which was nondialyzable and contained little sialic acid, 56 percent protein plus polypeptide including a fraction precipitated by heavy metals but dialyzable, and 4.7 percent non-protein nitrogen calculated as urea. About 50 percent of the ash could be accounted for as sodium, potassium, and chloride, while little or no inorganic phosphate could be detected. The protein nitrogen fraction contained marked enzyme activity of the acid phosphatase and proteolytic types. Ion-exchange cellulose chromatography of this fraction has yielded 15-fold purification of enzyme activities. Although the acid phosphatase fraction was devoid of proteolytic activity, no complete separation of a fibrinogenase and fibrinolysin could be obtained in any of the three clearly resolved proteolytic fractions after one chromatography passage. In the non-protein nitrogen group approximately ⅓ to ½ the nitrogen could be assigned to urea and ammonia.

A substance has been isolated that on analysis yields 24 percent nitrogen and gives a positive phenylhydrazine reaction. Traces of catechol amines occurred but no serotonin could be demonstrated by ultraviolet, colorimetric, or pharmacologic methods.

During this investigation of canine prostatic fluid, it was ascertained that a close relationship of dry weight, total nitrogen, and acid phosphatase content existed in their responses to hormone treatment. All parameters were increased by testosterone but declined during the administration of estrogen. After a critical dose of pilocarpine hydro-

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] Supported in part by Contract SA-43-ph-3761 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

chloride (0.7 mg/kg body weight) to produce secretion and a daily maintenance dose of testosterone (5 mg total dose per day) had been established, dogs with prostatic fistulas were subjected to estrogenic compounds at the 1 mg per day level for 10 days. Because of the relationship of acid phosphatase to the total nitrogen and dry weight mentioned, only the enzyme parameter in addition to volume was measured.

By dividing the maximum change in volume and acid phosphatase concentration, respectively, occurring during the test or posttest (delayed effect) period, by the mean of the pretest period, we could estimate the biopotency (table 1). There was generally good agreement between the mouse uterine assay and the canine prostatic secretion method in which estrogens decreased the fluid volume and enzyme content.

Table 1.—Comparison of relative potency of estrogenic substances by the mouse uterine and canine prostatic secretion bioassays* (estrone taken as 100)

Compound	Mouse uterine bioassay	Canine prostate bioassay	
		Total phosphatase	Volume and phosphatase concentration
Estradiol-17β, 17-ethynyl-	469	160	152
Estradiol-17β, 17-chloroethynyl-	420	206	227
Estradiol-17β	324	204	227
Estradiol-17β, 3-benzoate	333	45	140
Hexestrol	162	155	150
Diethylstilbestrol	157	181	182
Estrone	100	100	100
Estrone, KSO_4	88	60	70
Equilin	83	119	100
Hexestrol, -3-fluoro-	—	96	92
Estradiol-17β, 6-dehydro-	12	125	85
Estra-1,3,5(10)-trien-3-ol	13	36	45
Estrololactone	10	1. 5	1. 5
Estriol	5	6. 4	7. 5
Estradiol-17α	5	51	27

* Reprinted with permission from *Cancer Chemotherapy Reports* (*1*).

The question arose as to the usefulness of the dog assay if the only antiprostate effect was an inhibition of androgen expression. After a suitable number of nonestrogenic compounds was tested, it became clear that a variety of antiprostate reactions could be elicited. Thus it was important not to rely on volume changes alone.

With the measurement of 2 parameters, volume and acid phosphatase content, 9 possible combinations can occur. Seven of these took place, and probably an additional one, in which neither parameter was altered, but which was not obvious because the limits of the method are not known. In any case, it would appear highly suggestive that the canine prostatic secretion bioassay measured several antiprostate activities which may involve secretory function, water transport, and enzyme synthesis. Dehydration effects would be reflected by a fall in volume and a rise in phosphatase. An influence on water metabolism might explain a drop in volume with no change in phosphatase concentration. An unaltered

TABLE 2.—Influence of endocrine ablation on the levels of reducing systems and weight of the rat ventral prostate

Body weight (g)		Condition	Number of animals	Number of studies*	Formazan (μg per 100 mg wet weight)		
Mean	Range				Mean†	Range	Percent change from intact
109	(90–150)	Intact	70	7	122	(97–160)	
115	(60–160)	Adrenalectomized	40	4	169	(137–185)	+38
111	(96–122)	Hypophysectomized	22	2	80	(25–100)	−34
102	(65–132)	Adrenalectomized and hypophysectomized	14	2	56	(47–60)	−54
95	(63–120)	Castrated	240	24	57	(25–80)	−53
96	(66–129)	Castrated + 2.4 mg testosterone‡	240	24	193	(140–290)	+58
104	(62–119)	Castrated + 9.6 mg halotestin‡	25	5	137	(95–204)	+12

*Each study represents duplicate flasks for nontreated castrates and hypophysectomized rats, while triplicate to sextuplicate flasks were obtained on the other groups.

†Standard deviations were between ±6.1 to ±13.9 except for hypophysectomized and androgen-treated groups for which the value was close to ±22.0.

‡Testosterone was given subcutaneously and halotestin orally.

volume associated with a decline in phosphatase might suggest an inhibition of protein synthesis or zymogen release. With these considerations in mind, we examined the results obtained with 49 steroids and found obvious patterns in only 2 groups: Treatment with 12 of 16 estrone derivatives was associated with decreased volume and phosphatase content of the prostate, while treatment with 8 of 14 pregnane derivatives was associated with decreased volume and increased phosphatase content.

Any unification of hormone treatment and influence on the synthetic mechanism of the prostate could clarify the site of hormone action and reveal more pertinent parameters in bioassay procedures. Along these lines the dehydrogenase enzymes of the rat ventral prostate were investigated (table 2). Castration and hypophysectomy led to a drastic fall in enzyme content of the rat ventral prostate. This decrement could be restored by suitable hormone treatment. Parallel results were obtained in the presence and absence of succinic acid.

Additional work with triphenyltetrazolium is being carried out in bioassay procedures. One study (*2*) has revolved about the measurement of monoformazan in prostate slices of rats of varying ages (table 3). The titers of reducing systems reached a maximum at 28 days and declined to a plateau between 42 and 365 days. Histological observations on prostates from young and old, intact and castrated rats have been reported elsewhere (*3*).

Table 3.—Variation with age of reducing system titers in rat prostate

	Body weight (g)		Number of measurements		Formazan (μg per 100 mg wet weight)		
Age (days)	Mean	Range	Animals	Flasks	Mean*	Range	Percent change
15	27	(20–32)	20	2	107	(90–123)	
21	40	(36–43)	20	4	201	(195–214)	+88
28	72	(63–84)	20	8	276	(262–329)	+158
35	107	(65–132)	20	13	224	(181–257)	+109
42	140	(115–160)	20	14	176	(137–195)	+65
60	213	(150–287)	21	14	198	(164–220)	+84
90	323	(302–355)	20	10	171	(133–209)	+60
180	454	(370–491)	18	10	127	(111–161)	+19
365	554	(470–650)	20	18	180	(141–208)	+68

* Standard deviations were between ±18.1 to ±26.4 except in 21-day-old animals, ±8.8.

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COMMENTS ON ZINC IN THE HUMAN AND CANINE PROSTATES[1]

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The high zinc content of the human prostate was first reported in 1921 by Bertrand and Vladesco (*2*), who observed values of 0.49 and 0.53 mg of zinc per g dry weight in 2 human prostates. Not until 1951, with the observations of Mawson and Fischer (*18*) on zinc levels in various organs of the rat, did the subject of zinc concentration in prostatic tissue receive further specific attention. In 1952, Mawson and Fischer (*19*) reported an average zinc concentration of 0.859 mg of zinc per g dry weight in 7 normal human prostates and observed that benign hypertrophy, chronic prostatitis, and carcinoma of the prostate were associated with progressively lower zinc concentrations of 0.772, 0.332, and 0.190 mg of zinc per g dry weight, respectively. Hoare, Delory, and Penner in 1956 (*8*) found a mean zinc concentration of 0.744 mg of zinc per g dry weight and a range of 0.206 to 2.315 mg per g dry weight in 19 normal human prostates. Kerr *et al.* (*10*), in 1960, confirmed the high concentration of zinc in the human prostate and emphasized the marked variations in zinc concentration in different parts of the same gland. Daniel *et al.* (*5*), in 1956, and Prout *et al.* (*24*), in 1959, reported observations on uptake of zinc^{65} in human and canine tissues, suggesting a greater concentration of the isotope in the prostate than in other tissues assayed. Radioautography (*5*) and histochemical techniques (*25, 27*) localized the zinc in the epithelial portions of the prostate. Our observations (*7*), on zinc concentration in human prostatic tissue in general confirmed previous findings. Limitations in quality and quantity of human prostatic tissue available for zinc assay impair the validity of generalizations, but the following summary statements are at least consistent with available data:

(1) Zinc concentration in the normal human prostate averages approximately 0.8 mg per g dry weight.

(2) Zinc concentration in human benign prostatic hyperplasia is of the same order of magnitude as in the normal prostate gland.

(3) Zinc concentration in human prostatic cancer, both in untreated patients and in patients who have received endocrine therapy, averages approximately a third of the average normal level.

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(4) The normal over-all body average of zinc concentration in human soft tissues is about 0.1 mg per g dry weight.

Zinc concentration in the normal canine prostate is somewhat higher than in the human gland. In 1956, Weitzel *et al.* (*28*) reported the range of zinc concentration in the prostate was from 0.488 to 0.973 mg per g dry weight in 10 dogs, with an average zinc level of 0.677 mg per g. The zinc concentration in 6 dog prostates reported by Lo *et al.* (*12*), in 1960, ranged from 0.4 to 1.57 mg per g dry weight with an average of 1.25 mg per g dry weight. Mackenzie *et al.* (*15*) in a series of 68 mongrel dogs, including the 6 dogs of Lo *et al.* (*12*), observed that the range of prostatic zinc concentration was from 0.04 to 3.46 mg per g dry weight with an average concentration of 1.1 mg per g dry weight.

These observations make clear the high concentration of zinc in the human and canine prostates. In the human, the zinc concentration in the prostate is not exceeded by that of any other tissue except sperm, which contains as much as 2 mg per g dry weight (*21*). In the dog, however, in addition to high concentrations of zinc in sperm, retinal concentrations of up to 8 mg per g dry weight have been observed (*7*). Also, the low levels of prostatic zinc in the immature dog, the fall in prostatic zinc levels to about 10 percent of the precastration level following orchiectomy, the restoration of normal zinc levels in the prostate of castrated animals by the administration of testosterone, and the depression of prostatic zinc concentration following estrogen administration suggest the androgen dependence of prostatic zinc concentration (*14*). Other evidence of the androgen dependence of zinc concentration in the dog prostate is derived from studies suggesting an impaired zinc^{65} uptake in estrogen-treated animals (*23*, *24*). In man, although a similar dependence of prostatic zinc concentration on androgen probably exists, good evidence on this point is still lacking.

The reason for the high concentration of zinc in the prostate remains undefined. The high concentrations of zinc in human prostate, human seminal plasma (*20*), and human sperm cells (*21*) as well as in canine prostate, canine prostatic fluid (*15*), and canine sperm cells (*3*) imply that this zinc concentration is important in reproductive physiology. Gunn and Gould (*6*) demonstrated that the zinc of the dorsolateral prostate of the rat was not necessary for rat fertility or fecundity; but the finding by Wetterdal (*29*) of a high zinc^{65} concentration in the rat testis suggested that the high level of zinc in spermatozoa might well be independent of the prostate in this species, a possibility subsequently established by Birnbaum *et al.* (*4*). The remarkably high concentration of zinc in prostatic fluid obtained by digital massage of the prostate in 8 normal human subjects (average: 7.2 ± 1.9 mg zinc/g dry weight) (*16*), taken in conjunction with the low concentration of zinc in the human testis (*13*), the low zinc^{65} uptake in the human testis (*5*, *24*), and the high concentration of zinc in human spermatozoa, is consistent with the concept that a function of the prostate may be to provide sperm with zinc. A few observations demonstrating low zinc concentrations in the epididymal and vasal sperm from man and dog tend to support this hypothesis (*3*).

In 1952 Kadota (*9*) successfully produced a permanent diabetes in rabbits by the administration of dithizone (diphenylthiocarbazone), a chelating agent with an affinity for zinc. This observation led Kadota to suggest that the diabetes resulted from the chelation of zinc by dithizone in the *beta* cells of the islands of Langerhans. In 1960 Lo *et al.* (*12*) published observations on the effects of dithizone on the canine prostate and demonstrated that dithizone administration in dogs would result in a prompt cessation of prostatic fluid secretion, a decrease in zinc^{65} uptake in prostatic tissue, and a marked fall in zinc concentration in prostatic tissue. Dithizone also effected remarkable changes in the microscopic appearance of the prostate, including an initial proliferative response of abnormal prostatic epithelial cells followed by pyknosis and necrosis of many epithelial cells, followed later by an apparent atrophy but with ultimate restoration of the gland to a virtually normal appearance. These effects of dithizone administration are demonstrably dependent not only on the dose of dithizone but also on the rate at which the dose is given (*26*). Other effects of dithizone in the dog include blindness due to a lesion of the tapetum lucidum, the area of high zinc concentration in the canine retina, and an unexplained pulmonary edema and pleural effusion. It is the hope that dithizone or some other zinc chelating agent may find some application in the treatment of prostatic disease in man.

Although two reports (*11*, *22*) have suggested some beneficial effects of dithizone administration in patients with prostatic cancer, our own experience with 21 patients with proved advanced prostatic cancer in relapse, following conventional methods of therapy, demonstrated no objective benefit from using dithizone as we did. Two logical explanations for the lack of effect are: First, the dose of dithizone and the rate of administration in man have been limited by toxic effects—and it is possible that we have not achieved either a dose or a dose rate in man high enough to produce an effect; second, the zinc levels in human prostatic cancer are not sufficiently high to justify expectations of benefit. Studies (*17*) regarding the relationship of the zinc content of the canine prostate and the histologic effects of dithizone suggest that a higher concentration of zinc than we have observed in prostatic cancer must exist before one can anticipate lesions from dithizone as we have used it. Other chelating agents and other combinations of chelating agents are being explored (*1*) in the hope of resolving these difficulties.

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EXPERIMENTAL PROSTATIC TUMORS

Chairman: Olof H. Pearson

EXPERIMENTAL PROSTATIC CANCER: BACKGROUND OF THE PROBLEM[1, 2]

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Research in abnormal prostatic growth in man has been hampered by the lack of a suitable animal tumor. Benign enlargement occurs spontaneously in the senescent dog; approximately 80 percent of those over 8 to 10 years old exhibit some degree of hyperplastic change (*1*). However, histologically the process consists of a diffuse, cystic overgrowth, in contrast to the spheroidal type found in man. Although it is quite possible that the etiology of benign prostatic hyperplasia is the same in both species, the morphological characteristics and endocrine responses are quite different. Benign tumors are, unfortunately, rare in other species.

Prostatic cancer seldom develops in lower animals. In the dog the incidence is probably less than 1 percent, and spontaneous occurrence in other species is extremely rare. Only one has been reported in a primate (*2*) and none in rodents. This rarity may only be apparent, for, as Moore has pointed out, except for dogs most experimental animals either die or are killed in the laboratory during what amounts to childhood.

Carcinoma of the canine prostate, while comparatively uncommon, deserves some comment. It was described in association with pulmonary metastases as early as 1895 in the Norwegian literature. Occurring in older dogs, it is apparently highly malignant and metastasizes widely, but rarely to bone. Secondary osseous deposits have been reported only once, in an 11-year-old airedale. Canine prostatic cancer has no predilection for any particular breed. Histologically, it resembles its human counterpart quite closely. While fundamentally an adenocarcinoma, it may vary from a well-organized tumor to one composed chiefly of cords and nests of poorly differentiated cells (*3*).

Prostatic cancer in dogs does not seem to be associated with specific changes in hormone balance or those changes in the gland associated with aging. It has been found in glands exhibiting cystic hypertrophy, in

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others undergoing involution, and in others with both types of change. A prostatic cancer, in an otherwise atrophic gland, has been described in a 6-year-old fox terrier, in association with an adenocarcinoma of one testis involving both Sertoli and germinal cells (*4*). To my knowledge, endocrine therapy has never been used in a dog with prostatic cancer, although radiation has been reported to be ineffective.

Latent cancer of the prostate has not been found in the dog, though Goodpasture described changes suggesting it (*5*). This is the type noted by Franks in human specimens which, though morphologically identical with clinical cancer, nevertheless exhibits little or no growth activity (*6*), perhaps because of a lack of proper stimulus or the presence of a hypothetical inhibitor.

Experimental studies have been concerned with: (*a*) cultivation of human prostatic cancer in alien hosts (heterologous transplantation) or in tissue culture and (*b*) induction of prostatic cancer in lower animals or in normal prostatic tissue growing in culture.

EXPERIMENTAL STUDIES

Heterologous Transplantation

The first attempt to propagate human prostatic cancer heterologously was reported in 1945 by Masina, who used the anterior chamber of the guinea pig eye as originally described by Greene (*7*, *8*). Subsequent experience revealed that generally the percentage of successful takes was quite low, even with histologically proved cancer tissue from patients with metastases, who eventually died of the disease. Furthermore, though some transplanted tumors could be transferred indefinitely, they rapidly lost their acinar appearance and became frankly squamous with marked keratinization. Acid phosphatase was never present, and these tumors were from the beginning quite unresponsive to endocrine manipulation, including castration. Greene subsequently reported on the use of the brain as a site for heterologous transplantation (*9*,. However, Moreno and I (1950) were unsuccessful with this technique in obtaining takes of human prostatic cancer in guinea pig and rat.

The use of X radiation to condition animals for heterologous transplantation of human tumors was first described by Murphy in 1914 (*10*). In 1951 Toolan refined this technique with considerable success and subsequently added cortisone for further suppression of immunologic response (*11*). A variety of tumors has been successfully transplanted by Toolan's method, but, to my knowledge, not prostatic cancer. Schick and I (1951), using fresh prostatic cancer tissue obtained aseptically via open perineal biopsy, failed to achieve a single take in inbred rats or mice after many attempts with cortisone and X ray.

During 1960 and 1961 Nachbar and Shikes, two medical students working part time with me, employed a variety of other methods in an effort to develop a suitable experimental model for the heterologous transplantation of human prostatic cancer. These methods included the use of (*a*) chorio-allantoic membranes of chick embryos, (*b*) fetal and newborn mice of several inbred strains, primed with *in utero* or intraperitoneal inoculations of tissue suspensions or the buffy coat of donor blood, and (*c*) adult, inbred mice, pretreated with donor red blood cells, with and without testosterone. Both benign hyperplastic and malignant tissues were used, freshly obtained at surgery. With the foregoing methods only a few transitory takes were obtained. The circumstances under which this work was carried out were not entirely satisfactory, however, and the possibilities still seem promising enough to justify further study.

Tissue Culture of Human Prostatic Cancer

In 1917 Burrows, Burns, and Suzuki reported the successful propagation of human prostatic cancer cells (*12*). Since then, comparatively little has been reported on this subject. Recent studies have included those of Richter and Akin in 1957 (*13*) and Röhl in Sweden who, in 1959, described stimulation of such cultures following the addition of androsterone to the medium (*14*).

Induction of Experimental Prostatic Tumors

Most of the work in the field of experimental prostatic tumors has been concerned with attempts at induction in experimental animals or tissue culture.

Induction With Endocrine Substances

Both androgens and estrogens have been administered to animals in efforts to produce prostatic cancer, but without demonstrable success. Although androgens are necessary for active growth of normal or malignant prostate *in vivo*, there are no reports of prostatic cancer developing in experimental animals who have been given large doses of these compounds. Androgen administration has led to a diffuse type of benign prostatic hyperplasia in some experiments, but no actual nodules have been observed, either of the glandular component or the stroma.

Following estrogen administration to mice, squamous metaplasia occurs in the prostate, associated with some hyperplasia of the periurethral tissues. No bonafide carcinomas, however, have resulted, either of the acinar or epidermoid varieties (*1*). Lacassagne discovered extensive metaplastic changes in the posterior prostatic lobes of mice after large doses of folliculin given to induce breast cancer in males of a strain in which the females were highly susceptible to the disease (*15*). Burrows and Kennaway made similar observations following the application of ketohydroxyestrin in benzene to the skin of mice (*16*). Although this pronounced metaplasia has frequently been identified with epidermoid carcinoma, Moore found no evidence that the cells actually possess a neoplastic potential (*1*).

Induction In Situ

Following the pioneer work of Cook, Kennaway, and their co-workers with the carcinogenic hydrocarbons (*17-19*), a number of attempts were made to induce prostatic cancer in the rodent with these compounds. Moore and Melchionna in 1937 reported their work with 1 : 2 benzpyrene in lard, injected directly into the anterior prostatic lobes of white rats of unknown genetic constitution (*20*). They described a preliminary squamous metaplasia of the normal columnar epithelium, followed by malignant proliferation of the metaplastic cells. Tumors developed in 75 percent of the treated animals. No metastases occurred. Castration performed before or after appearance of the tumors had no effect either on tumor incidence or the rate of growth. Injections of testosterone propionate were of questionable value in increasing tumor incidence.

In 1946 Dunning, Curtis, and Segaloff described the induction of epidermoid carcinoma in the prostates of pure line rats by means of direct implantation of pellets of 20-methylcholanthrene (*21*). These tumors differed from those described by Moore and Melchionna in that they could be readily transplanted, and one of the tumors metastasized to bone, lung, and lymph nodes. Horning and Dmochowski injected 20-methylcholanthrene in lard into the anterior (ventral) and dorsal prostatic lobes of inbred mice and obtained transplantable squamous cancers which did not metastasize (*22*). In some of our early work we inserted minute quantities of pure 20-methylcholanthrene crystals directly into the ventral prostatic lobes of adult hooded rats and obtained nonmetastasizing squamous cancers. Although some of our tumors possessed a suggestive acinar appearance during the early stages, all subsequently became epidermoid.

Induction in Homologous Transplants

In 1945, Rous and Smith, while studying the neoplastic potential of mouse embryo tissues, first described their method for the rapid induction of skin cancer in mice of the C strain (*23*). This technique was later successfully adapted by these investigators to other tissues of the mouse, including prostate. Using this technique, Horning in 1946 reported the induction of a glandular carcinoma of the prostate from adult, not embryonic, tissue (*24*). Methylcholanthrene crystals were encased in strips of adult prostatic tissue from 8-week-old Strong A mice and transplanted subcutaneously to the abdominal walls of hosts of the same strain. In approximately 10½ weeks tumors developed in 12 of 14 mice, 11 of these tumors acinar and 1 squamous. Apparently no metastases occurred. These tumors proved readily transplantable. In 1949 Horning reported that transplants of his adenocarcinoma regressed in previously castrated mice (*25*). Growth was restored in a number of instances following the administration of testosterone propionate. Furthermore, diethylstilbestrol also seemed to retard the growth of this adenocarcinoma, but not as effectively as castration. In 1952 Horning found that methylcholanthrene induced tumors with greater frequency in mice pretreated with diethylstilbestrol than in untreated or androgenized mice (*26*). These findings, plus his observation that adenocarcinomas arose only from "resting" alveoli, led him to postulate that methylcholanthrene acted primarily on cells in a state of physiological exhaustion. This seemed to support Haddow's original contention that chemical carcinogens in general acted more effectively after depression of cellular activity (*27*).

Induction in Tissue Culture

In 1951 Lasnitzki cultured whole mouse prostate glands and found that 20-methylcholanthrene produced an increase in cell division, foci of anaplastic cells with abnormal mitoses, hyperplasia, and squamous metaplasia of the alveolar epithelium (*28*). In 1954 she reported that estrone caused epithelial hyperplasia in cultures of young glands and atrophy with stromal increase in older ones (*29*). In 1955 this investigator reported that young mouse prostate glands regressed when grown in normal control medium, but were able to retain their structure if testosterone propionate was added. After prolonged treatment such glands became hyperplastic. Older glands, on the other hand, were able to retain their normal structure in control medium, but became hyperplastic following the addition of testosterone propionate, with associated cytological and mitotic abnormalities. After prolonged exposure to the hormone, such glands became atrophic (*30*). There seemed, therefore, to be differences in the response of cells to estrogen as contrasted with androgen. Both hormones apparently were capable of affecting prostatic cells directly.

DISCUSSION

Although this area of investigation has not yet provided us with a dependable experimental tool, a number of promising leads have developed.

Spontaneously occurring prostatic cancer in the dog, while comparatively rare, looks and acts much like that in man. Just why it does not metastasize to bone is a matter of conjecture. Perhaps the explanation is a mechanical one, *e.g.*, related to venous drainage. In any case, canine cancer is worth more intensive study.

The glandular carcinoma developed by Horning in adult mice, unfortunately, has never been reproduced by other investigators. Ten years ago we studied a prostatic cancer originally induced with this technique by Smith in immature mice of the C strain. These tumors grew rapidly and were invasive, although they did not metastasize to distant sites. They were harvested every 6 to 8 weeks and transplanted for over 60 generations before the study was discontinued. Sections of this tumor revealed the characteristic histological pattern of epidermoid carcinoma of the transitional type. A number of areas bore an uncertain resemblance to Horning's tumors and human prostatic cancer, but were no different from similar areas observed in other squamous cell cancers.

Our tumors thrived equally well in male and female mice. In contrast to Horning's tumors, they were unaffected by castration or estrogen administration. Acid phosphatase was not found by the Gomori method.

It is conceivable that Horning's tumors arose from glandular epithelium before metaplasia to the transitional state had occurred. With the use of adult tissue containing cells with a slower growth momentum, and possibly also a slower rate of metaplasia, the carcinogen may have been able to exert its effect on the glandular cells longer than was possible in the more rapidly differentiating immature cells. This concept would be consistent with Haddow's original thinking about the increased carcinogenic effect on dormant cells. In all fairness we should state that Smith also used tissues from adult mice without being able to produce a convincing adenocarcinoma.

Along these lines the work of Lasnitzki offers some intriguing possibilities. Her results indicate that testosterone exerts a stimulatory effect similar to that of 20-methylcholanthrene on whole mouse prostates growing in culture. Furthermore, testosterone is more effective in cultures of older than in younger prostatic tissue, at a specific dose. Lasnitzki has suggested that ". . . malignancy may be caused by prolonged exposure of sensitive senescent epithelium to doses of androgen which are no longer physiological for the altered tissue." If this line of thinking can be transferred to human experiments, it may explain why prostatic cancer develops in the face of manifestly declining androgen levels in the body. It would be of great interest to study the effects of androgens—that is, testosterone and some of its better-known metabolites—in aging animals and in cultures of senescent prostatic tissue from animals and man. To my knowledge, there have been no recorded attempts to induce cancer in human prostatic tissue growing in culture.

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PROSTATE CANCER IN THE RAT[1, 2]

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Squamous cell carcinoma of the rat prostate has been induced by the direct application of benzpyrene (*1*) and methylcholanthrene (*2*). In both instances squamous metaplasia of the columnar epithelium was observed in contact with or in proximity to the inciting agent. Several of the neoplasms induced by methylcholanthrene have been successfully transplanted, and skeletal metastases have been observed in the primary hosts and in the rats bearing the subcutaneously transplanted tumors. Skeletal metastases are an unusual finding in rats bearing subcutaneously transplanted neoplasms. This affinity of prostatic tumor cells for bone was assumed to result from some inherent characteristic of the cell of origin. Further, no skeletal metastases were observed (*2*) when a metastasizing adenocarcinoma of mammary gland origin was successfully transplanted in the rat prostate.

The methylcholanthrene-induced carcinomas were obtained by the insertion of pellets of compressed carcinogen in the ventral prostate because it was more accessible in the rat than the dorsal or lateral lobes. Subsequent studies by Gunn *et al.* (*3*) and Gunn and Gould (*4*), however, showed that the dorsolateral lobes of the rat prostate were more functional in the uptake of Zn^{65} than was the ventral lobe, and the carcinomas induced in the ventral lobe proved to be nonfunctional in this respect.

Four transplanted methylcholanthrene-induced squamous cell carcinomas of the rat prostate were used for the assay of the carcinostatic potential of various steroids and other chemical agents. The results are summarized in tables 1 through 4 and shown graphically in text-figures 1 through 4. The following are the Cancer Chemotherapy National Service Center numbers and chemical names of compounds listed in tables 1 through 4:

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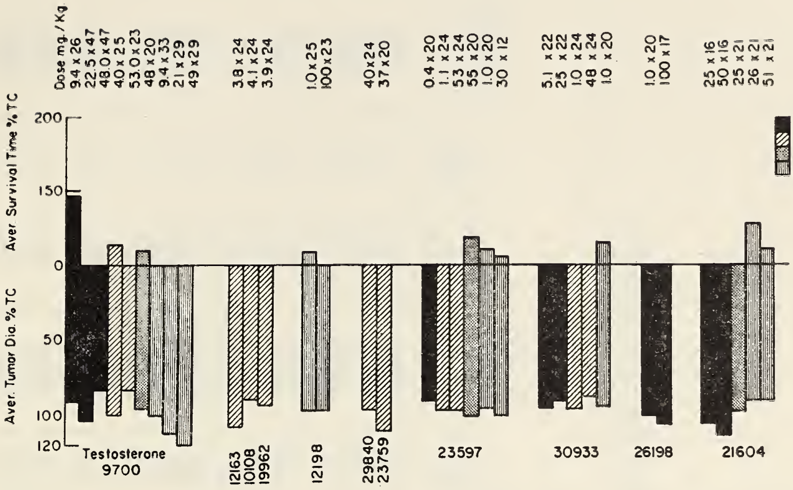
[2] Supported by contract SA-43-ph-3040 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

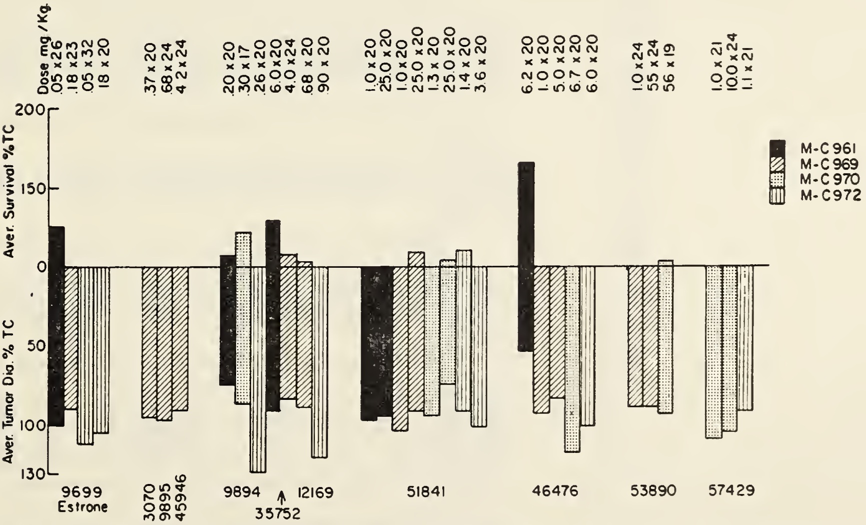
NSC NO.	CHEMICAL NAME
762	Diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride
3070	4,4′-Stilbenediol, α,α′-diethyl-
9699E	Estra-1,3,5(10)-trien-17-one,3-hydroxy-
9700E	Androst-4-en-3-one, 17β-hydroxy-
9704E	Pregn-4-ene-3,20-dione
9706	*s*-Triazine, 2,4,6-tris(1-aziridinyl)-
9894	Phenol, 4,4′-(1,2-diethylethylene)di-
9895E	Estra-1,3,5(10)-triene-3,17β-diol
10108	Ethylene, chlorotris (*p*-methoxyphenyl)-
12163E	Androst-5-ene-3β,17β-diol
12169E	Estra-1,3,5(10)-triene-3,16α,-17β-triol
12198E	5α-Androstan-3-one, 17β-hydroxy-2α-methyl-, propionate
17256E	Pregn-4-ene-3,11,20-trione, 6α-methyl-
19607E	Pregn-4-ene-3,11,20-trione, 2α-methyl-
19622E	Pregn-4-ene-3,20-dione, 21-fluoro-11β,17-dihydroxy-6α-methyl-
19962	3-Cyclohexene-1-carboxylic acid, 3-ethyl-4-(*p*-methoxyphenyl)-2-methyl-
21604E	5α-Androstan-17β-ol, 2α-methyl-
23597E	Androst-5-en-3-one, 17β-hydroxy-4,4,17-trimethyl-
23759E	17a-Oxa-D-homoandrosta-1,4-diene-3,17-dione
26198E	5α-Androstan-3-one, 17β-hydroxy-2α-methyl-
26386E	Pregn-4-ene-3,20-dione, 17-hydroxy-6α-methyl-, acetate
29840E	Estr-4-en-3-one, 17β-hydroxy-2α-methyl-
30933E	Androst-4-ene-3,11-dione, 9-chloro-17β-hydroxy-17-methyl-
35752	Phenol, 4,4′-(DL-1,2-diethylethylene)di-
45946	4,4′-Stilbenediol-α,α′-dimethyl-
46476E	Estra-1,3,5(10)-triene-3,16β-diol
48015E	A-Norpregn-3(5)-en-2,20-dione
48841	Malononitrile, (*p*-[bis(2-chloroethyl)amino]benzylidene)-
49520	*o*-Tolualdehyde,4-[bis-(2-chloroethyl)amino]-
50282	2-Biphenylamine, *N*- (4-[bis (2-chloroethyl) amino]-2-methylbenzylidene)-
50283	*m*-Toluidine,*N*,*N*-bis(2-chloroethyl)-4-*N*-cyclopentylformimidoyl-
51841E	Estra-1,3,5(10)-triene-16β,17β-diol,3-methoxy-
53890	Estr-5(10)-en-3-one, 17β-hydroxy-, acetate
53892	Progesterone, 11β,17-dihydroxy-6α-methyl-
56245	Quinoline,5-[(4-[bis(2-chloroethyl)amino]-2-methylbenzylidene) amino]-
56706	Aniline, *N*′, *N*′-bis(2-chloroethyl)-4-fluoro-2′-methyl-*N*,4′-methylidynedi-
57429	17β-Estradiol,17-(chloroethynyl)-
58251	Androst-4-en-3-one, 17β-hydroxy-6-methylene-, acetate
58799E	Pregn-4-ene-3,20-dione, 17-hydroxy-6-methylene-, acetate

Table 1 and text-figure 1 show for one or more of these tumors that in tests with testosterone and 10 related compounds there was no significant inhibition of the growth of the tumors, as measured by the comparative average diameters of the tumors in control and treated rats at the end of treatment. Likewise, in only two of the tests was there as much as a 20 percent increase in the survival of the treated rats compared with the untreated controls. In the majority of tests the untreated rats lived longer than the treated. The growth of one tumor (M-C 972) appeared to be stimulated by testosterone.



Text-figure 1.—Comparative response of 4 transplanted squamous cell carcinomas of the prostate to testosterone and related compounds, measured by the ratio of the average tumor diameter at the end of the treatment regimen in treated (T) and control (C) rats and by the ratio of the average survival time of the treated rats to that of the untreated.

Likewise from table 2 and text-figure 2 it may be seen that estrone and related compounds failed to inhibit the growth of these tumors significantly in spite of a considerable loss in body weight that resulted from the treatment in many instances. Only one test resulted in as



Text-figure 2.—Comparative response of transplanted squamous cell carcinomas of the prostate to estrone and related compounds, measured by the ratio of the average tumor diameter at the end of the treatment regimen in treated (T) and control (C) rats and by the ratio of the average survival time of the treated rats to that of the untreated.

Table 1.—Response of transplanted prostate carcinomas to testosterone and related compounds

					End of treatment						
Tumor/ generation	NSC number of compound	Dose per injection (mg/kg)	Number of daily injections	Number of rats (T/C)*	Change in weight in g (T/C)	Average tumor diameter in cm (T/C)	Percent (T/C)	Average survival in days (T/C)	Percent (T/C)	Percent toxic	Percent metastases (T/C)
M-C961/27A	9700E	9. 4	26	10/9	10/18	3. 1/3. 4	91	50/34	147	0	0/0
/35A	"	22. 5	47	7/7	4/15	2. 6/2. 5	104	32/66	48	0	0/14
/35A	"	48. 0	47	8/7	17/15	2. 1/2. 5	84	42/66	63	12	0/14
M-C969/42A	"	4. 0	25	9/10	5/12	3. 0/3. 0	100	43/37	116	0	0/0
26A	"	53. 0	23	10/10	6/11	2. 5/3. 0	83	42/44	95	0	60/10
M-C970/54A	"	48. 0	20	10/10	−2/2	2. 5/2. 6	96	40/36	111	0	20/10
M-C972/12A	"	9. 4	33	10/10	25/−2	3. 5/3. 4	103	48/56	86	0	0/0
/34A	"	21. 0	29	10/9	5/5	2. 7/2. 4	112	45/53	85	0	0/0
/34A	"	49. 0	29	10/9	17/5	2. 9/2. 4	120	46/53	86	0	0/0
M-C969/47A	12163E	3. 8	24	10/10	−54/−35	3. 1/2. 9	107	39/43	91	0	20/10
"	10108	4. 1	24	10/10	−44/−35	2. 6/2. 9	90	43/43	100	0	20/10
"	19962	3. 9	24	9/10	−64/−35	2. 7/2. 9	93	37/43	88	0	11/10
M-C972/45A	12198E	1. 0	25	9/9	−18/0	2. 2/2. 3	96	61/56	109	0	33/0
"	"	100. 0	23	9/9	0/0	2. 2/2. 3	96	56/56	100	0	67/0
M-C969/47A	29840E	40. 0	24	10/10	−37/−35	2. 8/2. 9	96	39/43	91	0	10/10
M-C969/46A	23759E	37. 0	20	10/10	−15/−9	2. 1/1. 9	110	36/43	84	0	0/10

M-C961/72A	23597E	0. 4	20	6/8	3/9	1. 6/1. 8	90	59/85	70	17	?/55
M-C969/51A	"	1. 1	24	10/10	−13/−4	2. 3/2. 4	96	32/39	82	0	10/20
"	"	53. 0	24	10/10	3/−4	2. 3/2. 4	96	30/39	77	0	0/20
M-C970/46A	"	55. 0	20	8/8	−34/−28	2. 9/2. 9	100	31/25	121	0	0/0
M-C972/48A	"	1. 0	20	8/10	− 7/−2	1. 8/1. 9	95	52/47	111	0	12/30
"	"	30. 0	12	10/10	15/−2	1. 9/1. 9	100	50/47	106	0	10/30
M-C961/81B	30933E	5. 1	22	8/10	−16/7	2. 0/2. 1	95	45/34†	132+	0	/0
/81B	"	25. 0	22	10/10	−3/7	1. 9/2. 1	90	47/34†	139+	0	/0
M-C969/51A	"	1. 0	24	10/10	−4/−4	2. 3/2. 4	96	35/39	90	0	0/20
"	"	48. 0	24	10/10	−6/−4	2. 1/2. 4	88	33/39	85	0	30/20
M-C972/51A	"	1. 1	20	8/9	−5/32	1. 9/2. 0	95	55/47	117	0	0/11
M-C961/68A	26198E	1. 0	20	8/7	8/7	1. 9/1. 9	100	45/53	85	0	25/28
"	"	100. 0	17	8/7	8/7	2. 0/1. 9	105	30/53	57	0	0/28
M-C961/81A	21604E	25. 0	16	7/3	−19/6	2. 2/2. 1	105	44/63	70	0	0/0
"	"	50. 0	16	7/3	−10/6	2. 4/2. 1	114	31/63	49	0	28/0
M-C970/65A	"	25. 0	21	9/10	−18/24	3. 4/3. 5	97	30/36	83	0	0/10
M-C972/56B	"	26. 0	21	10/10	−7/1	1. 6/1. 8	90	59/46	128	0	20/0
"	"	51. 0	21	10/10	−11/1	1. 6/1. 8	90	51/46	111	0	30/0

*T/C = treated/control.

†Treated tumor, rats alive to date.

Table 2.—Response of transplanted prostate carcinomas to estrone and related compounds

					End of treatment						
Tumor/ generation	NSC number of compound	Dose per injection (mg/kg)	Number of daily injections	Number of rats (T/C)*	Change in weight in g (T/C)	Average tumor diameter in cm (T/C)	Percent (T/C)	Average survival in days (T/C)	Percent (T/C)	Percent toxic	Percent metastases (T/C)
M-C961/27A	9699E	0. 05	26	10/9	−2/18	3. 4/3. 4	100	42/34	126	0	0/0
M-C969/26A	"	0. 18	23	10/10	−19/11	2. 8/3. 0	90	35/44	80	0	0/10
M-C972/12A	"	0. 05	32	9/10	−1/−5	2. 9/2. 6	111	48/56	86	0	0/0
/29A	"	0. 18	20	10/10	−12/6	2. 2/2. 1	105	57/65	87	20	0/0
M-C969/46A	3070	0. 37	20	10/10	−58/−9	1. 8/1. 9	95	42/43	98	0	10/10
/47A	9895E	0. 68	24	10/10	−70/−35	2. 8/2. 9	96	43/43	100	0	0/0
/47A	45946	4. 20	24	10/10	−51/−35	2. 7/2. 9	90	41/43	95	0	0/10
M-C969/46A	9894	0. 20	20	10/10	−47/−9	1. 4/1. 9	74	46/43	107	0	0/10
M-C970/12A	"	0. 30	17	10/10	−56/−41	2. 1/2. 4	88	35/29	121	0	10/0
M-C972/29A	"	0. 26	20	10/10	−21/6	2. 8/2. 1	130	57/65	88	0	10/0
M-C961/64A	35752	6. 0	20	9/10	−14/−3	1. 9/2. 1	90	47/36	130	0	11/20
M-C969/47A	"	4. 0	24	10/10	−62/−35	2. 4/2. 9	82	47/43	109	0	0/10
M-C969/46A	12169E	0. 68	20	10/10	−57/−9	1. 7/1. 9	89	45/43	104	10	20/10
M-C972/29A	"	0. 90	20	10/10	−41/6	2. 5/2. 1	120	51/65	78	10	0/10
M-C961/75A	51841E	1. 0	20	9/9	−26/17	2. 6/2. 7	96	34/50	68	0	0/0
"	"	25. 0	20	9/9	−44/17	2. 5/2. 7	93	28/50	51	11	0/0
M-C969/60A	"	1. 0	20	10/10	−47/−11	3. 1/3. 0	103	35/39	90	0	0/0
"	"	25. 0	20	9/10	−44/−11	2. 8/3. 0	90	43/39	110	0	0/0

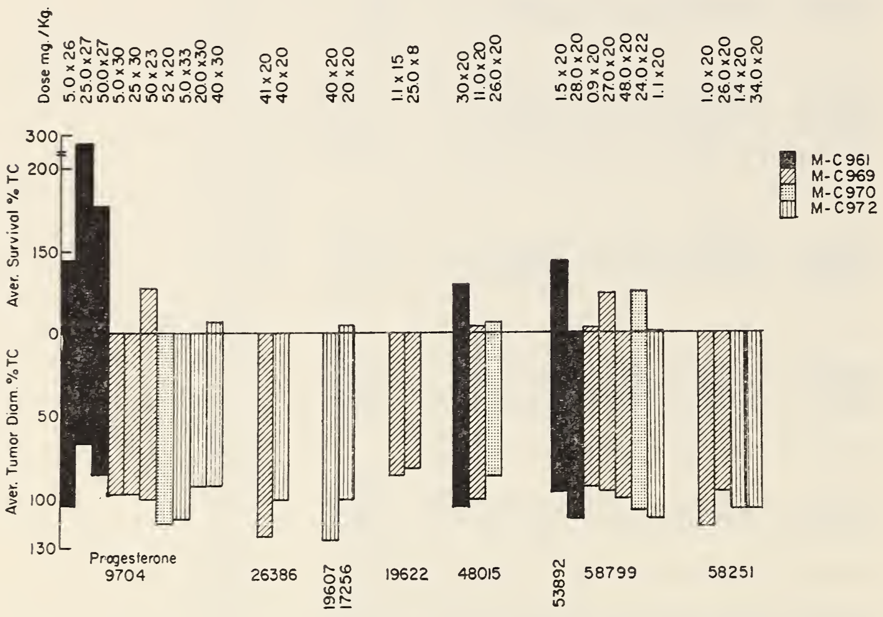
M-C970/57A	"	1. 3	20	10/10	−3/1	3. 3/3. 6	92	38/39	98	0	10/10
"	"	25. 0	20	10/10	−54/1	2. 6/3. 6	72	41/39	105	0	10/10
M-C972/52A	"	1. 4	20	9/10	−15/6	1. 8/2. 0	90	50/45	111	0	0/10
/52A	"	36. 0	20	10/10	−37/6	2. 0/2. 0	100	41/45	91	0	10/10
M-C961/73A	46476E	6. 2	20	2/3	−4/14	1. 0/1. 9	52	74/44	168	0	0/0
M-C969/54A	"	1. 0	20	10/10	−41/30	2. 3/2. 5	92	30/33	91	0	0/0
"	"	5. 0	20	10/10	−53/30	2. 2/2. 5	81	30/33	91	0	0/0
M-C970/52A	"	6. 7	20	6/7	−9/9	2. 6/2. 2	118	31/50	62	0	33/14
M-C972/49A	"	6. 0	20	9/9	−13/9	1. 8/1. 8	100	51/53	96	0	10/0
M-C969/51A	53890	1. 0	24	10/10	−28/−4	2. 1/2. 4	88	32/39	82	0	20/20
/51A	"	55. 0	24	10/10	−46/−4	2. 1/2. 4	88	38/39	97	0	10/20
M-C970/49A	"	56. 0	19	7/8	−17/9	2. 8/3. 0	93	28/27	103	0	?/?
M-C970/61A	57429	1. 0	21	10/9	−44/6	2. 6/2. 4	108	32/59	54	0	?/11
"	"	10. 0	21	10/9	−48/6	2. 5/2. 4	104	32/59	54	0	?/11
M-C972/56A	"	1. 1	21	10/10	−31/3	1. 6/1. 8	90	41/51	80	0	0/

* T/C = treated/control.

much as a 50 percent inhibition in growth of the tumors and a 68 percent increase in survival of the treated rats; this test, however, involved too few rats to be significant. The growth of the other 3 tumors was not inhibited by treatment with the same compound (NSC No. 46476E).

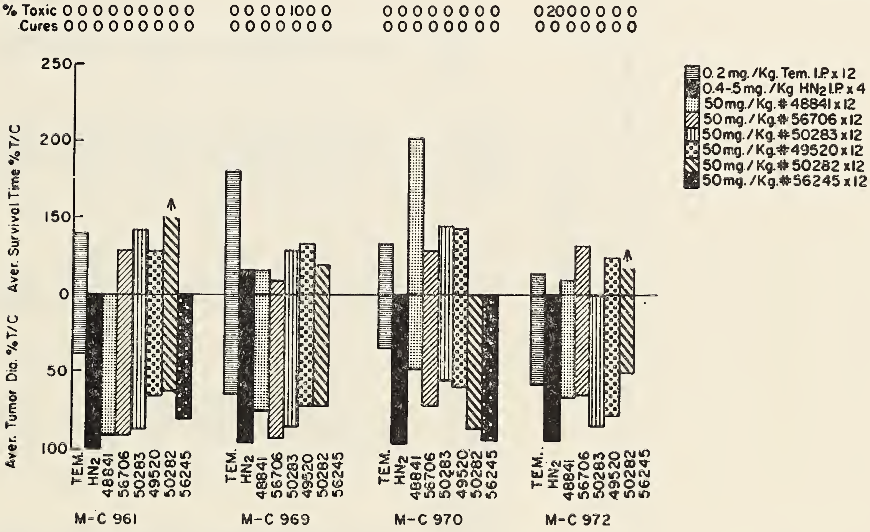
With the exception of M-C 961, these tumors were unresponsive to progesterone and the other steroids shown in table 3 and text-figure 3. The average survival of the progesterone-treated rats with M-C 961 tumors appeared to be increased, though with only 1 of 3 doses used, the average diameter of the tumors at the end of the treatment period was significantly reduced in rats that were treated.

Table 4 and text-figure 4 show the response of these 4 transplanted tumors to triethylenemelamine (TEM), nitrogen mustard, and a group of benzaldehyde mustards. The growth of all 4 tumors was somewhat inhibited by intraperitoneal injections of TEM. The drug was administered on an every-other-day schedule at 0.2 mg per kg for 12 injections. It was only slightly toxic at this dose level, and in each instance the survival of the treated rats was increased beyond that of the untreated. Nitrogen mustard, on the other hand, was ineffective when injected intraperitoneally at a maximum tolerated dose of 0.4 to 0.5 mg per kg on an every-4th-day schedule. The benzaldehyde mustards were admin-



Text-figure 3.—Comparative response of transplanted squamous cell carcinomas of the prostate to progesterone and related compounds, measured by the ratio of the average tumor diameter at the end of the treatment regimen in treated (T) and control (C) rats and by the ratio of the average survival time of the treated rats to that of the untreated.

istered subcutaneously in oil at approximately 50 mg per kg on an every-other-day schedule. On this regimen 5 of the 6 compounds cured or prevented the development of the isologously transplanted acute leukemia IRC 741. These tumors, like most isologously transplanted solid tumors, have been relatively unresponsive to all of the potential carcinostatic agents thus far tested. On this regimen the average survival of rats bearing M-C 961 was increased by treatment with 4 of the 6 compounds. The growth of M-C 969 and M-C 972 was slightly inhibited, and the average survival of the rats was increased by treatment with 4 of 5 benzaldehyde mustards. The growth of M-C 970 was inhibited, and the average survival of the rats treated with 4 of 6 of the compounds was increased. For one of the compounds (NSC No. 48841), the average survival time of the treated rats was increased twofold.



TEXT-FIGURE 4.—Comparative response of transplanted squamous cell carcinomas of the prostate to triethylenemelamine (TEM), nitrogen mustard, and a group of benzaldehyde mustards, measured by the ratio of the average tumor diameter at the end of the treatment regimen in treated (T) and control (C) rats and by the ratio of the average survival time of the treated rats to that of the untreated.

In August, 1961, a spontaneous tumor of the prostate was observed at necropsy in a Copenhagen male rat from the 54th brother × sister generation of line 2331. He was a 22-month-old retired breeder. The tumor occupied a large part of the lower abdominal cavity and appeared to involve primarily the dorsal prostate gland. It measured 5.0 × 4.5 × 4.0 cm and contained large cysts filled with semisolid gelatinous material. The noncystic tissue was pinkish white, translucent, and soft in consistency. Ten rats, 4 of the same inbred line as the host of the primary tumor and 6 F_1 hybrids from a Copenhagen × Fischer cross, were inoculated on the right side with 10 mg grafts of the soft tumor tissue and

TABLE 3.—Response of transplanted prostate carcinomas to progesterone and related compounds

					End of treatment						
Tumor/ generation	NSC number of compound	Dose per injection (mg/kg)	Number of daily injections	Number of rats (T/C)*	Change in weight in g (T/C)	Average tumor diameter in cm (T/C)	Percent (T/C)	Average survival in days (T/C)	Percent (T/C)	Percent toxic	Percent metastases (T/C)
M-C961/27A	9704E	5. 0	26	10/9	4/18	2.7/2.6	104	49/34	144	0	10/0
/37A	"	25. 0	27	8/9	4/17	2.4/3.5	68	84/30	280	0	0/0
/37A	"	50. 0	27	9/9	11/17	2.9/3.5	85	53/30	177	0	22/0
M-C969/39A	"	5. 0	30	10/10	−38/−31	3.2/3.3	97	39/40	97	0	0/0
/39A	"	25. 0	30	10/10	−31/−31	3.2/3.3	97	38/40	95	0	0/0
/28A	"	50. 0	23	10/10	13/19	3.1/3.1	100	36/28	128	0	0/0
M-C970/54A	"	52. 0	20	9/10	−27/2	3.0/2.6	115	27/36	75	0	22/10
M-C972/12A	"	5. 0	33	10/10	25/−5	2.9/2.6	111	54/54	100	20	0/0
/33A	"	20. 0	30	9/10	18/20	3.3/3.8	81	51/51	100	0	33/20
"	"	40. 0	30	10/10	13/20	3.3/3.8	81	55/51	108	0	20/20
M-C969/46A	26386E	41. 0	20	10/10	−19/−9	2.3/1.9	121	33/43	77	0	0/10
M-C972/41A	"	40. 0	20	9/6	−28/−19	1.2/1.2	100	71/74	96	0	53/33
M-C972/43A	19607E	40. 0	20	9/10	−10/−5	1.5/1.2	125	53/55	95	0	11/10
/57A	17256E	20. 0	20	7/8	−58/19	2.0/2.0	100	51/49	104	0	0/0
M-C969/52A	19622E	1. 1	15	10/10	−25/3	1.8/2.1	86	28/34	81	0	0/10
/52A	"	25. 0	8	10/10	−25/3	1.0/1.2	81	11/34	32	100	0/10

M-C961/70A	48015E	30. 0	20	6/7	4/2	2.3/2.2	105	85/66	130	0	50/28
M-C969/58A	"	11. 0	20	8/10	13/9	2.2/2.2	100	36/34	106	0	25/10
M-C970/53A	"	26. 0	20	10/10	8/−1	2.8/3.2	86	33/31	107	0	0/10
M-C961/67A	53892	1. 5	20	8/8	−7/12	2.4/2.5	96	46/32	144	0	25/12
/71A	58799E	28. 0	20	9/9	5/12	2.1/1.9	111	34/50	68	0	22/22
M-C969/56A	"	0. 9	20	10/10	−9/13	2.2/2.4	92	34/33	103	0	0/0
/55A	"	27. 0	20	8/9	−15/−22	2.1/2.2	95	39/31	126	0	0/11
/56A	"	48. 0	20	10/10	−7/13	2.4/2.4	100	33/33	100	0	0/10
M-C970/55A	"	24. 0	22	8/8	13/15	3.0/2.8	107	47/37	127	0	0/12
M-C972/50A	"	1. 1	20	8/8	5/1	1.9/1.7	112	47/46	102	0	12/12
M-C969/57A	58251	1. 0	20	10/10	−4/14	2.4/2.1	114	33/37	90	0	0/0
/57A	"	26. 0	20	10/10	3/14	2.0/2.1	95	32/37	86	0	0/0
M-C972/47A	"	1. 4	20	10/9	8/−5	1.7/1.6	106	49/51	96	0	10/11
/47A	"	34. 0	20	7/9	7/−5	1.7/1.6	106	48/51	94	0	14/11

*T/C = treated/control.

Table 4.—Response of transplanted prostate carcinomas to alkylating agents

Tumor/generation	NSC number of compound	Dose per injection (mg/kg)	Number of E.O.D.* injections	Site of injection	Number of rats (T/C) †	End of treatment							
						Change in weight in g (T/C)	Average tumor diameter in cm (T/C)	Percent (T/C)	Average survival in days (T/C)	Percent (T/C)	Percent toxic	Percent cures
M-C961/60A	9706	0. 20	12	ip‡	6/10	−23/0	0. 9/2. 2	39	84/58	140	0	0
M-C969/48A	"	0. 20	12	ip	10/10	−38/−37	1. 8/2. 8	64	56/31	180	0	0
M-C970/39A	"	0. 20	12	ip	8/9	−39/−34	1. 0/3. 0	33	39/29	134	0	0
M-C972/37A	"	0. 25	6	ip	8/10	−18/4	0. 8/1. 4	57	55/48	115	0	0
M-C961/61A	762	0. 4	4§	ip	8/10	−22/14	1. 7/1. 7	100	60/65	92	0	0
M-C969/49A	"	0. 5	5§	ip	10/10	−26/−6	2. 3/2. 4	96	39/31	116	0	0
M-C970/40A	"	0. 4	3§	ip	9/10	−35/−27	2. 3/2. 4	96	27/34	80	0	0
M-C972/38A	"	0. 5	4§	ip	10/10	−24/10	1. 5/1. 6	94	52/54	96	20	0
M-C961/76A	48841	52	12	sc‖	11/11	−2/9	1. 9/2. 1	91	48/81	60	0	9
M-C969/61A	"	44	12	sc	9/10	−15/0	2. 4/3. 2	75	38/33	115	0	0
M-C970/58A	"	47	12	sc	5/10	6/20	1. 5/3. 1	48	61/30	203	0	0
M-C972/54C	"	50	12	sc	8/8	−15/−1	1. 3/1. 7	77	56/51	110	0	0
M-C961/79A	56706	50	12	sc	9/9	−4/14	1. 9/2. 1	91	58/45	129	0	0
M-C969/62A	"	48	12	sc	10/10	−40/−27	2. 9/3. 1	94	41/38	108	0	0
M-C970/59A	"	50	12	sc	10/9	4/1	2. 3/3. 2	72	41/32	128	0	0
M-C972/55A	"	50	12	sc	9/10	−5/9	1. 6/2. 4	66	62/47	132	0	0

M-C961/77A	50283	51	12	sc	9/10	−23/10	2. 1/2. 4	88	65/46	141	0	0
M-C969/62A	“	48	12	sc	10/10	−36/−27	2. 7/3. 1	87	49/38	129	10	0
M-C970/59A	“	50	12	sc	6/10	−14/1	1. 8/3. 2	56	46/32	144	0	0
M-C972/55B	“	49	12	sc	9/10	−47/5	1. 7/2. 0	85	48/54	90	0	0
M-C961/80A	49520	52	12	sc	9/10	−13/11	1. 8/2. 7	67	56/44	127	0	0
M-C969/63A	“	52	12	sc	10/10	−32/−27	2. 3/3. 2	72	42/32	131	0	0
M-C970/60A	“	50	12	sc	7/10	2/14	1. 8/3. 0	60	56/39	144	0	0
M-C972/54A	“	50	12	sc	10/10	−21/13	2. 1/2. 7	78	58/46	126	0	0
M-C961/80A	50282	50	12	sc	6/10	−13/10	1. 7/2. 7	62	70¶/44	160+	16	0
M-C969/63A	“	52	12	sc	9/10	−17/−27	2. 3/3. 2	72	38/32	119	0	0
M-C970/63A	“	51	12	sc	8/10	−16/3	2. 7/3. 1	87	34/35	97	0	0
M-C972/58A	“	52	12	sc	8/8	−10/2	1. 1/2. 2	50	50/43¶	116+	0	
M-C961/80A	56245	55	12	sc	9/10	−6/10	2. 2/2. 7	81	36/44	82	0	0
M-C970/67A	“	50	12	sc	9/10	−20/5	2. 3/2. 4	96	34/41¶	83	0	0

*E.O.D. = every other day.
† T/C = treated/control.
‡ ip = Intraperitoneal.
§ Every 4th day.
‖ sc = Subcutaneous.
¶ Some rats alive to date.

on the left side with somewhat larger quantities of the gelatinous material. Representative sections were preserved for microscopic examination. The primary tumor was classified as a papillary adenocarcinoma as shown in figures 1 and 2. No metastases were identified.

The transplanted tumors grew very slowly and became palpable on the 60th day on the right side of each of the inoculated rats. No tumors appeared at the site of injection of the gelatinous material on the left side of the rats. One rat was killed for identification of the tumor and transplantation on the 60th day, and another tumor was excised and transplanted on the 134th day. Sections of these tumors are shown in figures 3 and 4. On the 245th day another tumor from the first transfer generation was dissected, frozen, and preserved for study by Dr. Gunn and Dr. Gould. They reported that histologically the tumor showed glandular formation with a great deal of cellular material corresponding to normal dorsal-type glands of the rat prostate. The tumor tissue showed positive periodic acid-Schiff (mucopolysaccharide) and negative dithizone stain typical of dorsal-type glands. The electrophoretic pattern also was characteristic of dorsal gland secretion. Gunn and Gould concluded that histologically and histochemically this tumor showed characteristics indicating that it originated from the dorsal component of the dorsolateral prostate. A sample of heart blood from one rat with a tumor from the first transplanted generation showed an acid phosphatase level of 9.5, which was not as high as has been observed (14.4, 16.2, and 20.9) in some of the rats bearing the transplanted squamous cell carcinomas of the prostate.

Tumors of the second transplanted generation will soon be available for an assay of their response to possible chemotherapeutic agents. Dr. Gunn and Dr. Gould are making more comprehensive functional studies of some of the tumors from the second transfer generation that will be reported subsequently. It is hoped that the rate of growth of the tumor may be increased by selective transplantation and that it may prove useful for screening therapeutic agents.

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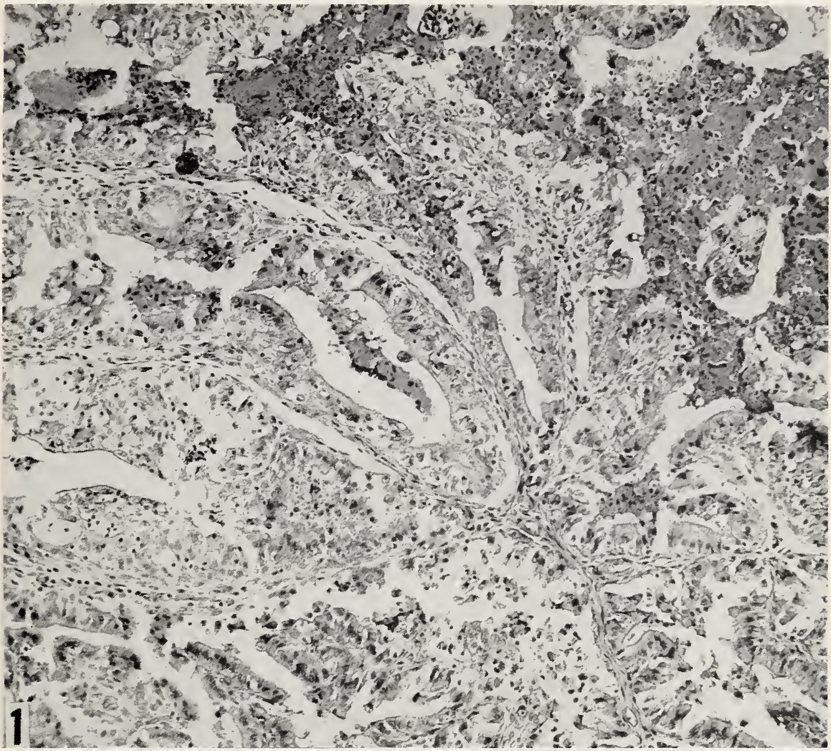
PLATES

Plate 35

Figure 1.—Section of papillary adenocarcinoma of the prostate R3327/0 to 1A. × 109

Figure 2.—Section of R3327/0 to 1A papillary adenocarcinoma of the prostate. × 581



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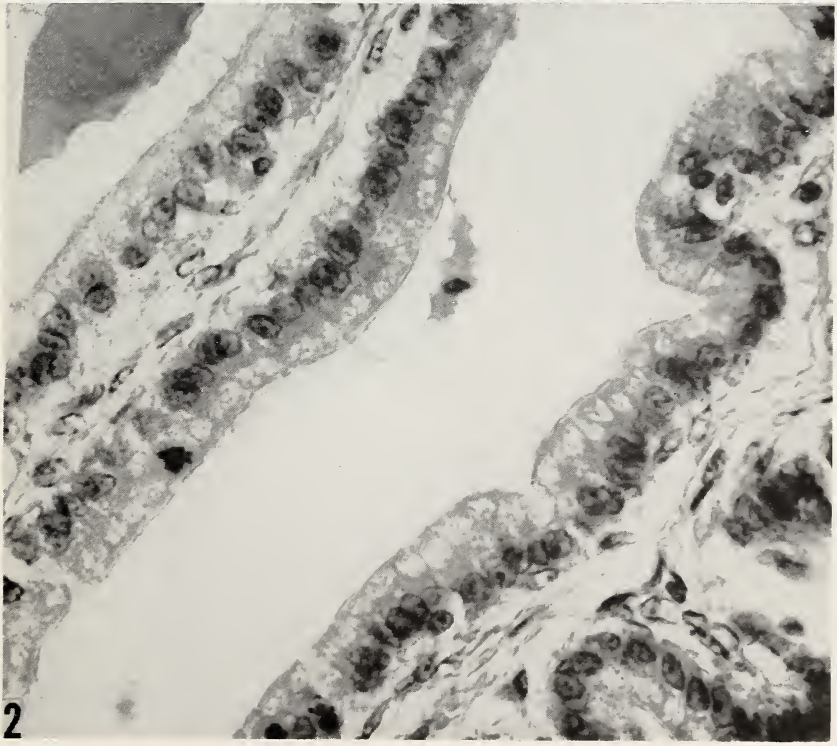

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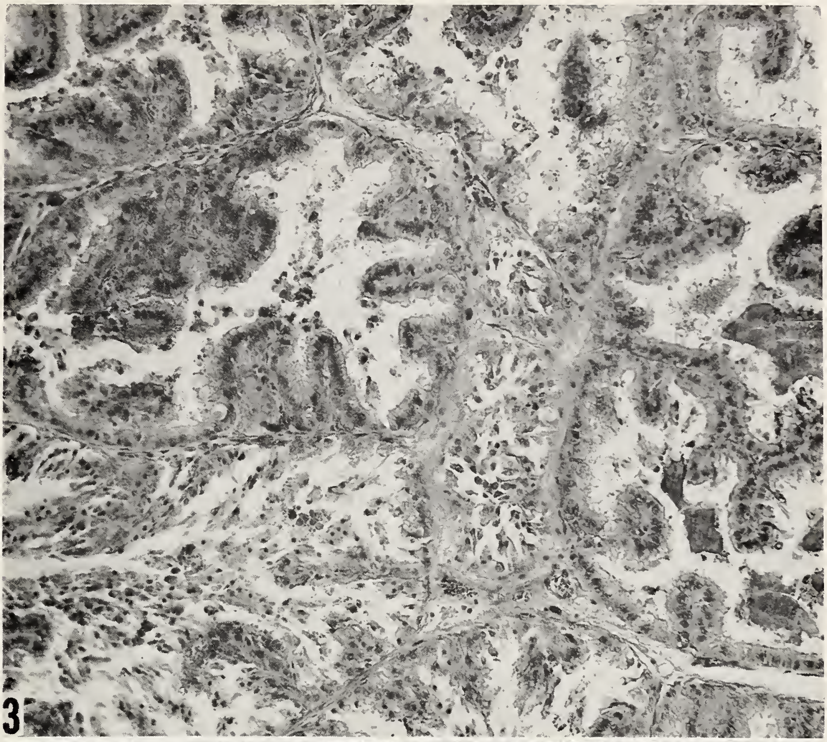
Plate 36

Figure 3.—Section of papillary carcinoma of prostate R3327/1A to 2C, 134 days after subcutaneous transplantation. × 109

Figure 4.—Section of R3327/1A, 60 days after subcutaneous transplantation, with papillary carcinoma showing osteometaplasia. × 109



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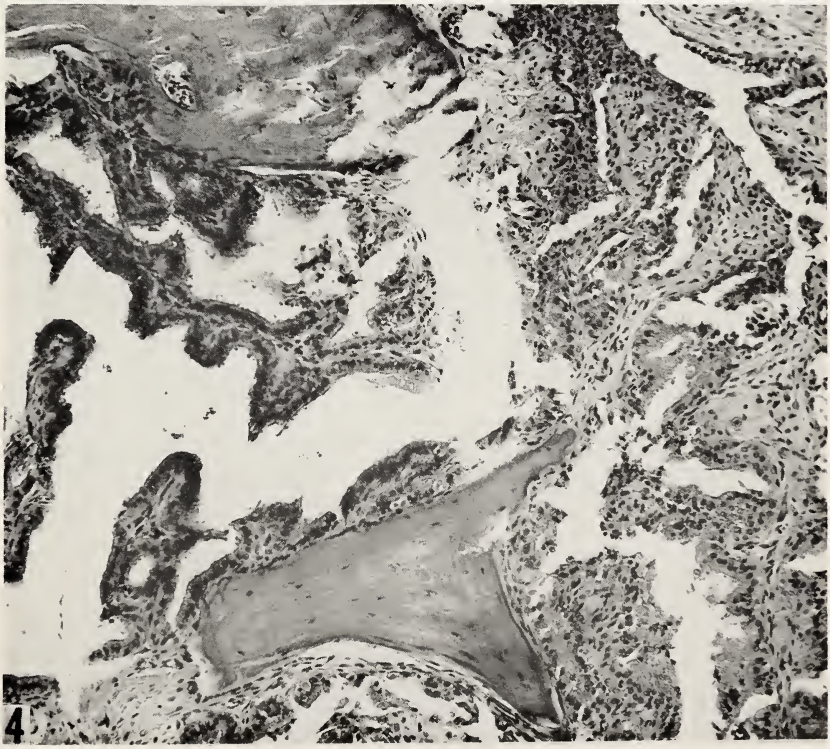

4

A TRANSPLANTABLE, SPONTANEOUS ADENOCARCINOMA OF THE PROSTATE IN THE SYRIAN (GOLDEN) HAMSTER [1,2]

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MAN and dog (*1–3*) are ordinarily considered the two species that spontaneously develop prostatic cancer. Recently, Fortner (*4*) noted an adenocarcinoma of the prostate gland in 2 of 94 untreated hamsters varying in age from 9.5 to 35 months. The neoplasms seem to be one manifestation of an unknown factor causing a wide variety of spontaneous tumors in the Syrian hamster.

Successful attempts to induce prostatic cancer in laboratory animals have usually resulted in squamous carcinomas rather than adenocarcinomas (*5, 6*). On three occasions, however, adenocarcinomas of the prostate gland have developed in animals subjected to 3-methylcholanthrene. Horning (*7, 8*) induced transplantable neoplasms in mice of a STR-A inbred strain by wrapping small isolated strips of prostatic epithelium around a few crystals of pure carcinogen. A high incidence of adenocarcinomas resulted when this preparation was transplanted subcutaneously in host animals of the same strain. Mirand and Staubitz (*9*) placed crystals of methylcholanthrene in the ventral prostate of 99 Wistar rats. The prostate was the site of an adenocarcinoma in 2, of a squamous carcinoma in 30, and of a leiomyosarcoma in 3 animals. Unfortunately, the adenocarcinomas were not transplanted. Kallen and Rohl (*10*) implanted a mixture of methylcholanthrene in a cholesterol-glycerin vehicle into the ventral lobe of 9 adult albino rats of the homozygotic R strain. One rat developed an adenocarcinoma which grew in tissue culture.

A spontaneous and transplantable adenocarcinoma of the prostate in a laboratory animal has not been previously reported. The evident need for such an experimental tool prompts the present report.

MATERIALS AND METHODS

The tumor-bearing animal described in this report was one of an untreated group of hamsters obtained from the Lakeview Hamster

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

[2] Supported by grant C–03817 from the National Cancer Institute, National Institutes of Health, Public Health Service.

[3] The authors are indebted to Dr. Stephen S. Sternberg for his invaluable help in the histologic studies and to Dr. Theodore Hall for the zinc determinations.

Colony in Newfield, New Jersey. The animals had been maintained under ordinary laboratory conditions and were fed a diet of Purina laboratory chow with a slice of fresh carrot daily. Tap water was given *ad libitum.* The animal was allowed to live until natural death. Tissues from the original animal and from animals bearing the transplanted tumor were fixed in 10 percent formalin and stained with hematoxylin and eosin for histologic study.

Transplantation utilized the mince suspension technique with implantation of tumor into the subcutaneous tissue of animals about 6 weeks old (*11, 12*).

The zinc concentration of transplantable tumors and of the prostate of normal hamsters was determined through the courtesy of Dr. Theodore Hall, using the method of X-ray fluorescence (*13*). The acid phosphatase content of the peripheral blood of normal hamsters and of tumor-bearing hosts was determined. Blood from 3 to 7 animals was pooled in order to obtain a sufficient volume for single determinations. The tissue acid phosphatase content of the normal hamster prostate and of the transplantable tumor was also studied. Serum and tissue acid phosphatase determinations were performed in the Biochemical Department of Memorial Hospital; a modification of the Bodansky method (*14*) was utilized.

RESULTS

Primary Tumor

A 21-month-old male hamster of an untreated group died on August 10, 1960. At necropsy, the hamster weighed 116 g. A large tumor in the pelvis replaced the area of the prostate gland. There was invasion of the abdominal wall on the left and of the bony pelvis.

The neoplasm was pink-tan and showed central necrosis. Metastases were present on the serosa of the colon and in the lungs. Other grossly evident abnormalities were enlargement of both adrenal glands and of Cowper's gland on the right. Two small cysts of the liver and subcutaneous edema were present.

Transplantable Tumor

A portion of the primary tumor was transplanted subcutaneously into male hamsters. Fifteen days later, the first generation tumor was transplanted into both males and females. Growth was comparable in the two sexes. The tumor was then transplanted at intervals varying from 6 to 20 days until the 27th transplant generation. At this time and subsequently, it has been transplanted at 7-day intervals. The transplantable tumor, designated as Prost. #1, was in the 93d transplant generation on September 26, 1962. The implanted tumor takes in 100 percent of animals and grows well in males and females. The tumor is moderately firm, gray-white, and has a hemorrhagic center with some central necrosis (fig. 1). Occasionally, enlarged axillary lymph nodes are noted. After 7 days, the tumor begins to ulcerate, with death of hosts occurring about 2 weeks after transplantation.

Ascitic Form

A mince suspension of a sixteenth generation tumor was inoculated intraperitoneally. Ascites was evident in about 2 weeks. For the first few generations, the ascitic form was transplanted every 2 weeks. At about the tenth passage, a 7-day transplant interval was initiated and maintained. Hosts die about 10 days after inoculation of 0.5 cc of ascitic fluid (fig. 2). At this time, marked ascites and cachexia are evident. The ascitic fluid is bloody, and tumor is grossly apparent on the peritoneal surfaces. The ascitic form of Prost. #1 was in the 69th transplant generation on September 26, 1962.

Histologic Studies

The hamster's prostate gland is divided into both dorsal and ventral lobes and morphologically is similar to the prostate of the mouse and rat (*15*). Figure 3 shows the histologic appearance of the prostate of a normal hamster which can be compared with the appearance of the primary prostatic neoplasm in figures 4 and 5. It is evident that the tumor is a moderately undifferentiated adenocarcinoma with foci of papillary formation. Individual cells vary greatly in size and show nuclear chromatin clumping and large, prominent nucleoli. Mitotic figures are prominent. Both acinar and papillary differentiation is present. These histologic features are retained in the pulmonary metastases which were evident grossly. Tumor cells were not found in multiple sections of dorsolumbar vertebrae and the long bones. The sacrum was directly invaded by the pelvic tumor. The animal also had a low-grade cystadenocarcinoma of a Cowper's gland and bilateral nodular, adrenocortical hyperplasia. The seminal vesicles appeared normal.

Tumors in various transplant generations have retained a morphology similar to the original (fig. 6). Host bearing the transplantable tumor developed lymph node (fig. 7) and pulmonary metastases. Unconcentrated smears of ascitic fluid from the ascitic variant of Prost. #1 contain numerous free-floating cancer cells (fig. 8).

Chemical Studies

The zinc concentration of a representative sample of Prost. #1 was 0.15 mg per g dry weight of tissue. The ventral prostate of a 5-month-old hamster contained 0.084 mg and the dorsal prostate 0.16 mg of zinc per g dry weight of prostate. In a 6-week-old hamster, the ventral prostate had a zinc content of 0.10 mg and the dorsal prostate 0.11 mg per g dry weight. A transplantable Cowper's gland cystadenocarcinoma, which is carried in our laboratory, had a zinc content of 0.067 mg per g dry weight.

This may be compared with zinc values for normal Cowper's glands of 0.083 mg for the 5-month-old hamster and 0.1 mg for the 6-week-old hamster. The zinc content of a given tissue, according to Hall, varies from animal to animal by about 10 percent.

The tissue acid phosphatase content of two representative samples of Prost. #1 was 123.5 and 139 Bodansky units, respectively, per 100 g of wet tissue. For comparison, the acid phosphatase values for two grossly normal prostate glands were 107.5 and 95.5 units per 100 g of wet tissue.

Pooled sera from 12 groups of hamsters bearing established Prost. #1 tumors contained an average of 3.77 units of acid phosphatase. Similarly, pooled sera from 9 groups of normal male hamsters contained an average of 4.1 units. Four groups of normal females had an average of 3.4 units of acid phosphatase.

DISCUSSION

The primary cancer reported here was so extensive that the exact prostatic lobe from which it originated could not be determined. It has been demonstrated that the prostate gland is not a homogeneous structure with respect to its functional activity, chemical or enzymatic concentrations, or in its response to estrogens (*16–19*). Failure to find significant elevations of serum acid phosphatase in hosts bearing Prost. #1 may be due to the tumor's origin in a chemically and functionally silent area of the prostate. The apparent lack of hormone dependency of the tumor may be related to its etiology or to its dedifferentiated morphology.

The zinc content of the transplantable tumor was similar to values obtained for the prostate gland. This contrasts with the findings in man in whom a decreased concentration of zinc is found in prostatic cancer as compared with normal prostate. Kerr, Keresteci, and Mayoh (*16*) found the zinc content of the human prostate to vary from 0.478 to 0.726 mg per g dry weight. These values are substantially higher than those obtained for the hamster.

Tissue acid phosphatase values for the hamster prostate were lower than those reported by Woodard (*20*) for the human prostate. Prost. #1 had a somewhat higher acid phosphatase content than normal hamster prostate. In the human, the acid phosphatase values are lower in the cancerous prostate than in the normal prostate.

SUMMARY

Prost. #1 is a transplantable adenocarcinoma derived from a spontaneous adenocarcinoma of the prostate gland of the Syrian (golden) hamster. The tumor shows uniform takes in both male and female hamsters. Chemical analysis reveals the zinc content to approximate

that of normal hamster prostate tissue. Tissue acid phosphatase values for the tumor are somewhat higher than those for the hamster prostate. No elevation of the serum acid phosphatase values of hosts bearing the tumor was found.

The transplantable prostatic cancer, in both solid and ascitic forms, is available to interested investigators.

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PLATES

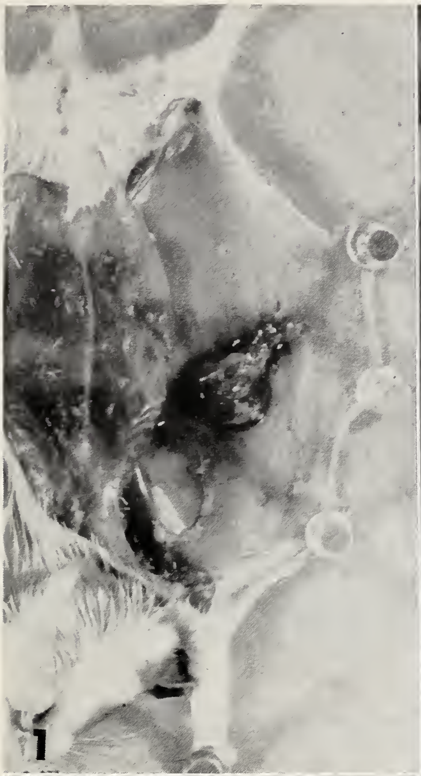
PLATE 37

FIGURE 1.—Gross appearance of Prost. #1, 7 days after implantation.

FIGURE 2.—Distended abdomen of hamster with the ascitic form of Prost. #1.

FIGURE 3.—Normal prostate of hamster. × 200

FIGURE 4.—Primary prostatic adenocarcinoma. Urethra is in the center with adenocarcinoma in the surrounding prostate. × 100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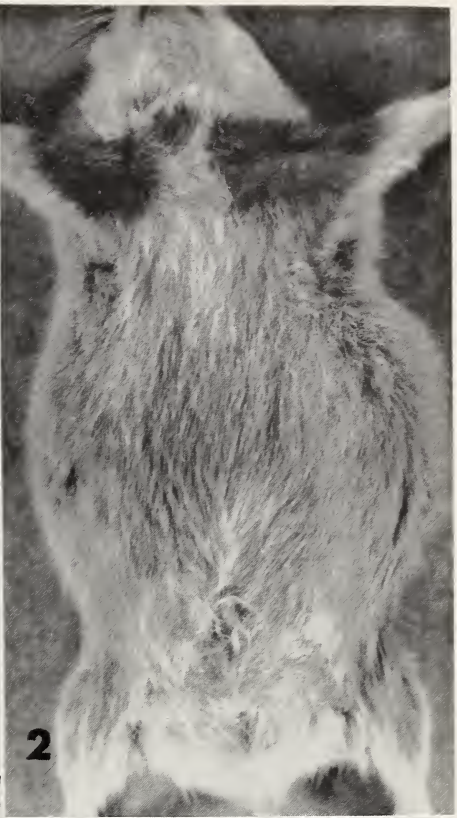
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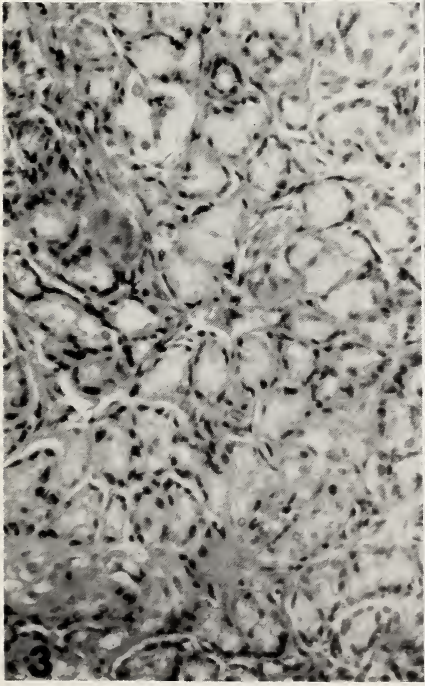
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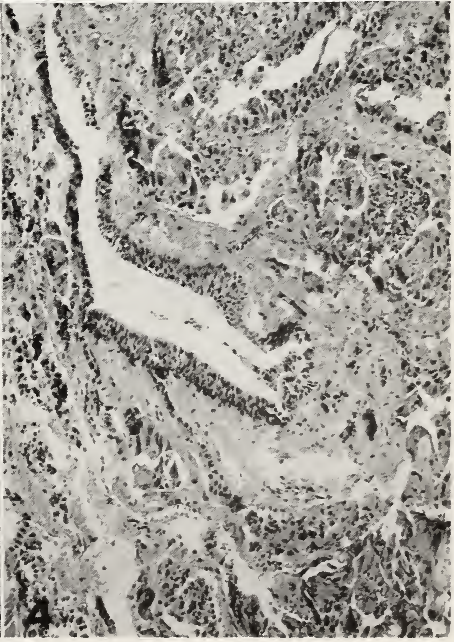
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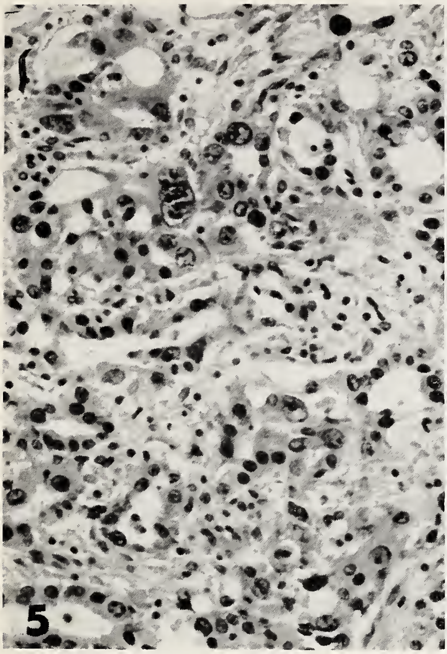
PLATE 38

FIGURE 5.—An area in the primary tumor. × 200

FIGURE 6.—Prost. #1 in the 37th transplant generation. Glandular pattern persists, desmoplastic reaction is marked. × 200

FIGURE 7.—Focus of metastatic cancer from Prost. #1 in a peripheral sinus of lymph node. × 150

FIGURE 8.—Cancer cells in an unconcentrated smear of the ascitic form of Prost. #1. × 440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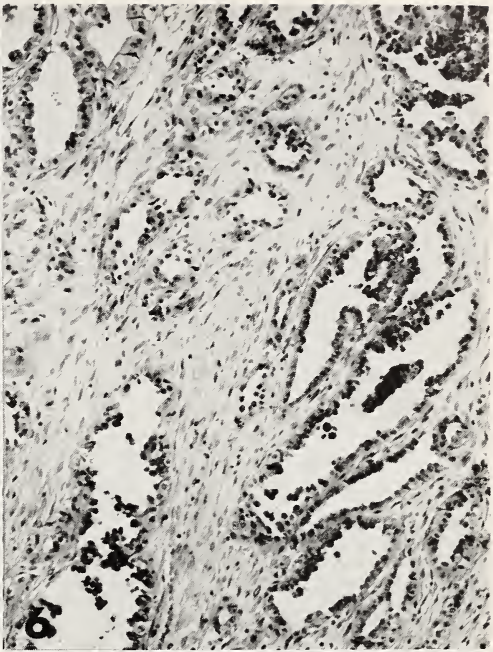
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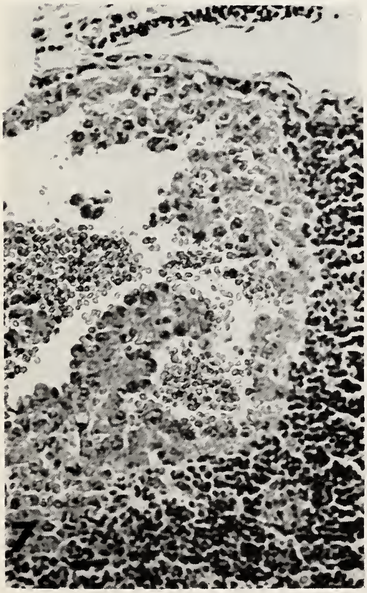
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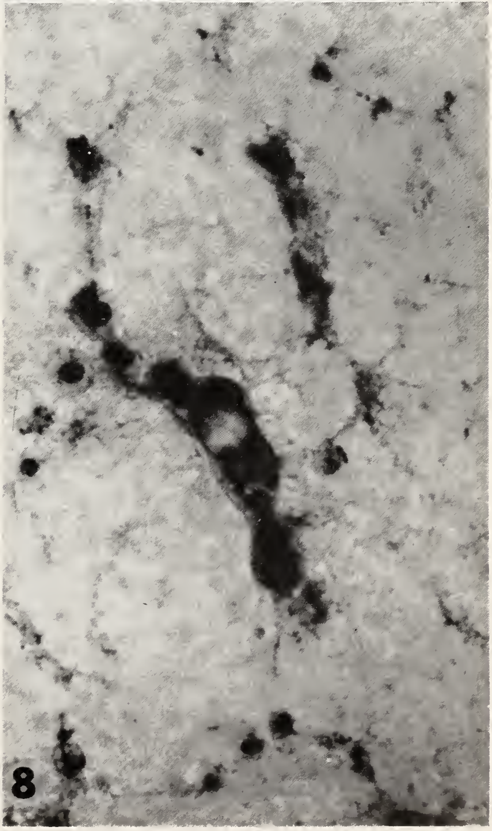
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GROWTH PATTERN OF THE MOUSE PROSTATE GLAND IN ORGAN CULTURE AND ITS RESPONSE TO SEX HORMONES, VITAMIN A, AND 3-METHYLCHOLANTHRENE[1]

ILSE LASNITZKI,[2] Strangeways Research Laboratory, Cambridge, England

The tissue culture method offers many advantages for the study of the physiology and pathology of the prostate. In animal experiments the response of the organ to environmental changes is a result of a complex reaction in which the effects of physical or chemical agents are modified by general systemic factors and the intervention of the endocrines. But in the isolated organ both these factors are eliminated, complicated problems can be broken down into their elements, and the question decided whether the effect of a given compound is due to a direct effect on the tissue. Histological and biochemical changes can be detected and followed from an early stage and the interaction of several different substances studied in detail. Cultures are also a convenient tool to correlate morphological with biochemical changes.

In this paper the growth pattern of the gland of different ages in normal medium and its response to sex hormones are examined; the effect of a carcinogenic hydrocarbon, 3-methylcholanthrene, and modification of its effect by sex hormones and vitamin A are investigated; and the changes due to vitamin A deficiency of the medium are described.

MATERIAL AND METHODS

The ventral prostate glands obtained from C3H mice, 4 to 6 weeks, 3 months, and 5 to 6 months of age, and anterior prostate glands from 3-month-old mice of the same strain were used for this study.

The glands were excised under sterile conditions and freed from the surrounding sheath of fat and connective tissue. The two lobes of the ventral gland were separated, and one was explanted as experimental culture while the other served as control. The larger anterior gland was subdivided into fragments not exceeding 2 × 3 × 4 mm in size, and comparable explants were grown as control and experimental cultures.

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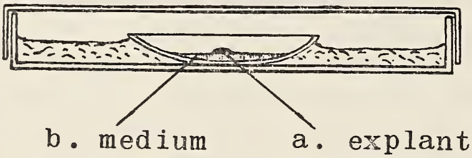
[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] Sir Halley Stewart Fellow.

To preserve the histological structure of a tissue, it is essential to place it on the surface of the culture medium. The glands were, therefore, grown by the standard watchglass technique (*1*) on the surface of a clot consisting of a mixture of cock plasma, horse serum, and chick embryo extract, or on strips of rayon acetate (*2*) placed on the clot. For the use of fluid media the organ was explanted on squares of lens paper (*3*) that floated on the medium. The medium was contained in a watchglass which rested in a petri dish carpeted with damp cottonwool (text-fig. 1). Estrone and testosterone propionate (Organon, London), 3-methylcholanthrene, and vitamin A alcohol (Roche) were incorporated into the medium before it clotted. The explants were transferred to fresh medium every 3 or 4 days and removed for histological examination or biochemical assays after various periods of growth ranging from 5 to 25 days.



TEXT-FIGURE 1.—Watchglass technique.

RESULTS

The ventral prostate in vivo consists of ducts and alveoli lined with cuboidal or columnar secretory epithelium surrounded by muscle and collagen fibers. Periodic acid-Schiff-positive secretory matter can be seen (fig. 1) as a thin film at the apical surface of the cells or as more solid deposits in the lumen. The epithelium is either straight or slightly folded and occasionally reserve cells can be recognized between it and the basement membrane. In glands from 3- and 5- to 6-month-old mice the epithelial nuclei or the whole cell is occasionally pyknotic and is shed into the lumen.

The anterior prostate in vivo also consists of alveoli and ducts surrounded by thin fibromuscular stroma. Most alveoli are filled with papillary projections consisting of rows of columnar cells mounted on either side of a connective tissue base, whereas in the ducts the epithelium is either straight or only slightly folded (fig. 2).

Growth Pattern *In Vitro*

The gland maintains its general histological structure *in vitro*, and alveoli surrounded by fibromuscular stroma can be recognized as in the organ before explantation. But there are variations in the degree to which the epithelial cells and their secretory function are preserved and in the amount of stroma, which depend on the age of the glands and also whether they have been grown in air or in oxygen.

Ventral glands from young mice (4–6 weeks old) showed some atrophic changes when grown in air in a medium without added hormones. After 10 days' growth the epithelium is reduced in height, the folding is lost, and secretion ceases. New alveoli are formed at the periphery of the explant, but these are straight and lined with one layer of cuboidal or more flattened epithelium. This atrophy is accompanied by a considerable increase of the stroma, which has become richer in cells and fibers (fig. 7).

In ventral glands from mice 3 to 6 months old and in anterior prostates from 3-month-old mice grown in air without added hormones, the structure is much better preserved *in vitro*. The histological pattern as seen under the light microscope resembles that of the organ *in vivo* except for the central regions (figs. 3 and 4). Both the pre-existing and the newly formed alveoli at the periphery show slightly folded cuboidal or columnar epithelium that continues to secrete, though at a reduced rate as compared with the gland *in situ*. In the central areas the epithelium is often low or atrophic and in some alveoli it breaks down and is shed. This suggests that essential metabolites or oxygen may not reach the central regions in sufficient quantities. A comparison of cultures grown in air and in an atmosphere of 95 percent O_2 and 5 percent CO_2 showed that perfusion with the latter gas mixture prevented the central necrosis. In addition, the height and folding of the epithelium were increased further, and the level of secretory activity was restored to that of the gland *in vivo* (figs. 5 and 6).

Studies on the amino acid requirements of prostatic explants from 3-month-old mice by means of exchange chromatography of the medium before and after cultivation of the gland (*4*) showed that the glands precultured for 9 to 10 days utilized leucine, isoleucine, cystine, and large amounts of arginine while glutamic acid and glycine accumulated (table 1) Ornithine, not present initially, was found in the medium at the end of

TABLE 1.—Utilization of amino acids by mouse prostate tissue in medium 1118

Amino acid	Unused medium (mg/liter)	Medium from control cultures (10.2 mg wet weight; mg/liter)
Aspartic acid	58. 4	57. 2
Threonine	31. 8	31. 7
Glutamic acid	160. 0	202. 0
Glycine	97. 0	104. 0
Alanine	47. 2	35. 6
Cystine	142. 0	126. 0
Valine	47. 0	43. 3
Methionine	22. 9	15. 9
Isoleucine	41. 5	29. 8
Leucine	119. 0	85. 7
Tyrosine	37. 8	36. 0
Phenylalanine	48. 2	51. 5
Arginine	90. 5	Trace

TABLE 2.—Effect of cultivation on the arginase activity of mouse prostate tissue

	μg Urea produced per 100 mg wet weight per hour	μg Urea produced per 100 μg DNAP per hour
Ex vivo tissue	437	
Control cultures	2, 041	
Ex vivo tissue	516	1, 560
Control cultures	10, 900	53, 350
Ex vivo tissue	348	903
Control cultures	7, 306	13, 976
Testosterone-treated cultures	6, 508	23, 300

the experiment in quantities approximating the yields obtainable from the breakdown of arginine by arginase action. Estimations of arginase activity by Brown and Cohen's method (*5*) showed that the activity was 12 to 35 times higher than that of the tissue directly isolated *ex vivo* (table 2). Addition of testosterone propionate to the medium did not prevent this rise—a finding that rules out lack of androgenic hormones in the medium as a causative factor. In this connection Westfall *et al.* (*6*) found that two strains of mouse fibroblasts derived from a single cell had 20 and 400 times, respectively, the arginase activity of the parent strain.

The difference in growth pattern observed between young (fig. 7) and older glands in medium without added hormones suggested that the young organ depends on a continuous supply of androgenic hormones which are apparently not available in sufficient quantities in the culture medium, while older glands are independent of it. This possibility was tested by the addition of testosterone propionate to the medium for the growth of young and old glands (*7*).

Effect of Hormones

It was found that explants from young mice grown for 10 days with 50 μg per ml of testosterone propionate retain their structural differentiation and resemble glands of the same age *in situ* (fig. 8). If the treatment is continued, however, the alveolar epithelium becomes hyperplastic. After 3 weeks' growth, most of the alveoli become partially or completely occluded by increased proliferation of the lining epithelium. Four to eight rows of densely packed cells bordered internally by a single row of secretory epithelium can be distinguished. These show a great number of normal cell divisions and regular enlargement due to an increase of the cytoplasm (fig. 9). The hormone also reduces the increase of the intra-alveolar stroma seen in the untreated controls.

In contrast, addition of the same dose of testosterone propionate to the medium with glands from 5- to 6-month-old mice stimulated an intense multiplication of the alveolar epithelium shortly after the beginning of treatment. After 10 days' growth the hyperplastic epithelium consisted of crowded cells of irregular size, among which many abnormal cell divisions could be recognized (fig. 10). Prolongation of the treatment resulted in atrophy and widespread necrosis of the epithelium in most explants.

These results show that the hormone affects the prostatic epithelium directly, and that glands from young mice require additional hormones to preserve their structure while explants from older mice are either hormone-independent or need a much lower concentration for their maintenance.

The finding that a dose of hormone, which was sufficient to maintain young glands, induced hyperplasia in the older organs indicates that the older epithelium has not become refractory but rather more sensitive to the hormone. This idea is supported by the appearance of degenerative changes in the older organ after longer periods of hormone application. Apart from the mitotic abnormalities and the irregular increase in cell size, which were observed in the older glands only, the difference in response is mainly one of degree since the older glands undergo epithelial hyperplasia after prolonged treatment.

This is in contrast to the response of the gland to estrone, which is basically altered depending on the age of the explanted organ. It was demonstrated (*8*) that estrone added in doses of 2 to 4 μg per ml to the medium of young glands induced multiplication of reserve cells, which led to extensive hyperplasia and squamous metaplasia of the alveolar epithelium within 10 to 28 days' growth (fig. 11). Similar changes have been described by Horning (*9*) and Bern *et al.* (*10*) for prostate glands of young mice and rats injected with estrogens.

Addition of estrone to the medium of older glands, on the other hand, caused a flattening and atrophy of the alveolar epithelium accompanied by hypertrophy of the fibromuscular stroma particularly after the higher doses (fig. 12). The alveoli became surrounded by a wide, dense zone of collagenous and muscle fibers, and stromal elements filled the interalveolar spaces absorbing and replacing necrotic alveoli in the process.

The greater sensitivity to testosterone and the reversal of the estrone effect from a synergist to an antagonist are difficult to interpret, but suggest that the enzymatic equipment of the gland may change radically during its development.

The responsiveness of the gland to testosterone was partially confirmed by Franks (*11*) and Franks and Barton (*12*), who found that the hormone increased the height of the epithelium, prevented degenerative changes in the mitochondria seen in glands cultured without testosterone, and evoked a secretory cycle. However, in their experiments the stimulation of secretory activity was confined to the glands from 5-month-old mice. In young glands the hormone produced a slight epithelial atrophy with an increase of stroma and muscle. Estrone and estradiol failed to stimulate the young gland but produced a graded atrophy of the epithelium with increase of the fibromuscular stroma in both young and older glands. The failure of the hormone to stimulate the young gland may be due to differences in susceptibility of the strain of mice used or to differences in the composition of the medium. Thus the medium used by Franks may have contained a larger amount of estrogenic or gonadotrophic hormones, which would have modified the effect of the added testosterone or estrogens. The use of a hormone-free defined medium in place of the biological medium would rule out this complication.

Effect of Vitamin A

We were also interested in the influence of vitamin A on the growth pattern of the gland (*13*). This vitamin plays an important role in the maintenance of secretory epithelia. Vitamin A deficiency usually causes squamous metaplasia of secretory epithelia *in vivo* (*14*) while exposure to excess vitamin A of squamous epithelia *in vitro* and *in vivo* results in their mucous transformation (*15, 16*). We found that excess vitamin A applied to ventral prostate glands of 3-month-old mice, grown in biological medium, did not materially alter their pattern of growth nor increase their secretory activity. But cultivation in a completely defined medium not containing the vitamin resulted in the production of widespread, irregularly distributed foci of squamous metaplasia after 10 days *in vitro*. The squamous changes were frequently but not always preceded by increased proliferation of reserve cells. In some alveoli, horn pearls at various stages of development could be recognized beneath an intact secretory epithelium (fig. 13); in others the covering epithelium broke down and was replaced by squamous cells that deposited dense strands of keratin into the lumen. In such alveoli the lining may alternate between squamous and normal glandular regions (fig. 14). Other alveoli were lined with one or two rows of flat cells and occluded with masses of keratin (fig. 15). This effect differs distinctly from that observed after treatment with estrone, which was always associated with a marked hyperplasia and where the changes involved the whole of the alveolar lining in any one alveolus. The two types of metaplasia thus correspond to the distinction made by Fluhmann (*17*) between "prosoplasia," a term proposed by Fischer-Wasels (*18*) to designate a more orderly, generalized squamous transformation seen after hormonal stimulation in many organs, and the irregular multifocal metaplasia following trauma or vitamin A deficiency. It was found that addition of vitamin A to the defined medium completely prevented the squamous changes (fig. 16), which indicated that the changes were, in fact, due to A hypovitaminosis of the cultured prostatic tissue.

Prostatic cancer in man usually occurs in the elderly or aged. The difference in hormone sensitivity with age found in our experiments in the mouse prostate raised the question whether the changed response to hormones is a factor in the formation of such tumors.

We decided to investigate this problem by growing mouse prostate glands of various ages in the presence of 3-methylcholanthrene. Horning (*9, 19*) obtained glandular and squamous carcinomas in ventral prostates from young mice treated with methylcholanthrene—an indication that the gland is sensitive to this carcinogen. It was seen that the carcinogen incorporated into the culture medium affected the gland directly and provoked changes of a precancerous nature (*20, 21*), closely resembling the early stages of carcinogenesis *in vivo* described by Horning (*19*). In the cultures the substance produced mitotic stimulation which led to a marked hyperplasia of the alveolar epithelium within 5 to 10 days' treatment. The newly formed hyperplastic epithelium consisted of small cells of the reserve cell type that partially occluded the lumen and which were lined with a layer of secretory epithelium (figs. 17, 18, 19, and 20). In some cultures hyperplasia of an adenomatous type could be recognized due to increased formation of small secreting alveoli (fig. 21). In explants carried on for a further 10 days without the carcinogen, the changes progressed and the hyperplasia became more extensive.

The hyperplasia was always associated with a loss of differentiation. The secretory lining epithelium broke down and was not replaced; instead the hyperplastic cells showed irregular enlargement (fig. 22) or formed clusters without organization, in which a great number of abnormal mitotic figures could be observed (fig. 23). In most explants, however, the cells became stratified and underwent squamous metaplasia (figs. 24 and 25). The changes described were identical in glands from young and older mice and seemed, therefore, independent of the age of the explanted organ.

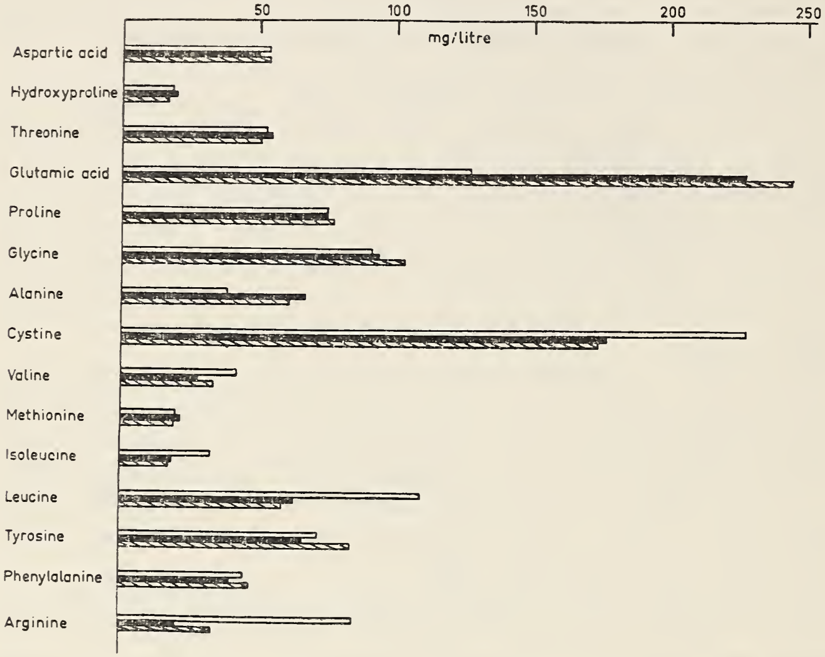
Since vitamin A inhibits squamous development of epithelial tissues, it was thought possible that it might also prevent the squamous metaplasia seen after treatment with methylcholanthrene in the prostate gland.

We found that simultaneous application of the vitamin with the carcinogen did not interfere with the growth-promoting action of methylcholanthrene; it did, in fact, inhibit the squamous transformation and preserve the secretory lining epithelium (*22*).

In such cultures, the hyperplastic epithelium consisted of small heaped cells of the reserve cell type (figs. 26 and 27) or the columnar secretory cells became associated with cuboidal cells with a basophilic cytoplasm connected with each other and with the secretory elements by tonofibrils. In contrast to the squamous alveoli, where mitosis was confined to the basal layers, these cells remained potentially dividing, and mitotic figures could be seen in all strata. Hence the hyperplasia was usually more extensive than in explants treated with the carcinogen alone.

Administration of the vitamin after withdrawal of methylcholanthrene not only suppressed the squamous transformation but also was followed by a partial reversal of the hyperplasia so that there were fewer hyperplastic alveoli per explant, and in these the hyperplasia was less extensive (fig. 28). This reduction of hyperplasia may have been due to an increased uptake of the vitamin in the absence of competition from the carcinogen. It was not caused by a direct damaging effect on the hyperplastic epithelium nor was it due to an inhibition of cell division, since excess vitamin was found to increase the mitotic rate in cell cultures (*23*). Possibly the vitamin controls the rate of cell multiplication and differentiation in secretory epithelia and restores the normal balance of the two processes.

Measurements of amino acid utilization of carcinogen-treated and untreated explants (*4*) showed that both series utilized the same amino acids and that the quantities taken up were of the same order of magnitude, with the exception of arginine, which was removed more slowly from the medium by cultures grown with 3-methylcholanthrene (text-fig. 2). This was found to be due to a lower level of arginase since the carcinogen partially inhibited the striking increase of the enzyme observed in the controls (table 3).



TEXT-FIGURE 2.—Amino acid utilization in medium BLI by mouse prostate glands grown with and without 3-methylcholanthrene. □ = Unused medium; ■ = used medium from control cultures; and striped square = used medium from 3-methylcholanthrene-treated cultures. [Lasnitzki and Lucy (*4*).]

TABLE 3.—Effects of cultivation and 3-methylcholanthrene on the arginase activity of mouse prostate tissue

	Urea, μg, produced per 100 mg wet wt tissue per hour			Urea, μg, produced per 100 μg DNAP per hour
Ex vivo tissue	425	437	12	—
Control cultures	3, 920	4, 980	1, 060	—
3-Methylcholanthrene-treated cultures		2, 041		—
Ex vivo tissue		516		1, 560
Control cultures		10, 900		53, 350
3-Methylcholanthrene-treated cultures	1, 460	1, 731	271	7, 480

Effect of Estrogen and 3-Methylcholanthrene

Estrogens used in the therapy of prostatic cancer in man caused a temporary regression of primary and secondary lesions (*24, 25*); in methylcholanthrene-induced glandular carcinomas of the prostate of young mice, treatment with estrogens or orchiectomy retarded tumor growth (*9*). But estrogens applied together with methylcholanthrene during carcinogenesis accelerated and increased the production of squamous prostatic tumors (*19*). It is not clear whether this effect is due to a direct action on the cells or is brought about by the restriction of gonadotrophic hormones in the pituitary gland. The organ culture of the prostate seemed a convenient tool to clarify this point and to study the interaction of methylcholanthrene and estrogen at the cellular level. It was found (*8*) that simultaneous application of methylcholanthrene and estrone to young glands (2 μg/ml and 1 μg/ml estrone) accelerated the squamous transformation as compared with cultures treated with the carcinogen alone (figs. 29 and 30). However, the same combination applied to older glands produced a pattern of response similar to that seen after the hormone alone: inhibition of epithelial growth and stimulation of the fibromuscular stroma (fig. 31). Thus the modification of the carcinogen effect by the hormone seemed determined by the age of the gland and produced a similar pattern of response as that seen after the hormone alone.

On the other hand, application of estrone to young glands previously treated with methylcholanthrene caused a widespread destruction of the hyperplastic epithelium. The cells enlarged, the cytoplasm became vacuolated and the nuclei pyknotic (fig. 32). In areas showing advanced hyperplasia, whole clusters of cells were shed, leaving empty alveoli behind. These degenerative changes closely resembled those described by Horning (*9*) in induced carcinomas of the mouse prostate following treatment with diethylstilbestrol and also those seen by Fergusson and Franks (*26*) in human prostatic cancer following estrogen therapy. This similarity suggests that the hormone affects the cancer cells directly, although an additional effect via the endocrines cannot be ruled out.

The difference in effect between simultaneous and successive application of the hormone corresponds to that observed by Horning (*9, 19*) after administration of the hormone during carcinogenesis and after establishment of the tumors.

SUMMARY AND CONCLUSIONS

The experiments show that both sex hormones and 3-methylcholanthrene affect the mouse prostate gland directly on the cellular level and that this effect resembles that seen after administration of the compounds *in vivo*.

It is seen that the sensitivity of the gland to sex hormones changes during advancing age: The young gland requires additional testosterone for its full maintenance, while the older gland is less dependent on it. This is a quantitative difference. In contrast, the response to estrone is more basically altered from hyperplasia and squamous metaplasia of the alveolar epithelium in the young organ, to epithelial atrophy and stimulation of the fibromuscular stroma in the older gland.

The functional maintenance of the gland also depends on the presence of vitamin A in the medium. Cultivation in a defined medium in the absence of the vitamin provokes the formation of irregularly distributed squamous-keratinizing foci, which can be prevented by addition of vitamin A.

The induction of precancerous changes by 3-methylcholanthrene and its modification by vitamin A are not dependent on the age of the animal used, but the influence of estrone on the carcinogenic effect follows the pattern seen after the hormone alone.

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PLATE 39

FIGURE 1.—Section through ventral prostate, from a 3-month-old mouse, before explantation. Periodic acid-Schiff (PAS)-positive. × 115

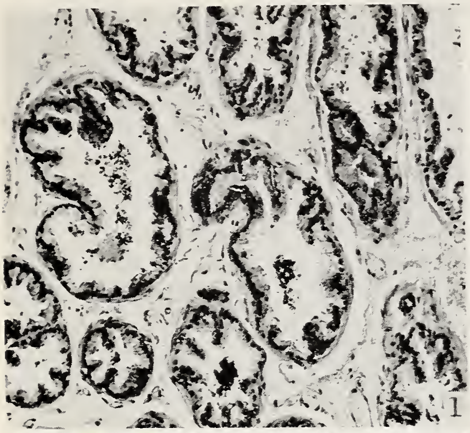
FIGURE 2.—Section through anterior prostate, from a 3-month-old mouse, before explantation. PAS-positive. × 115

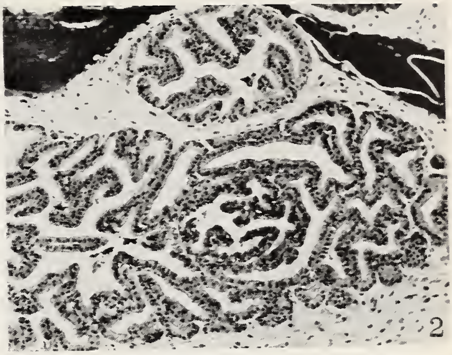
FIGURE 3.—Section through ventral prostate, from a 3-month-old mouse, cultivated for 10 days in air, showing well-preserved alveoli. PAS-positive. × 115

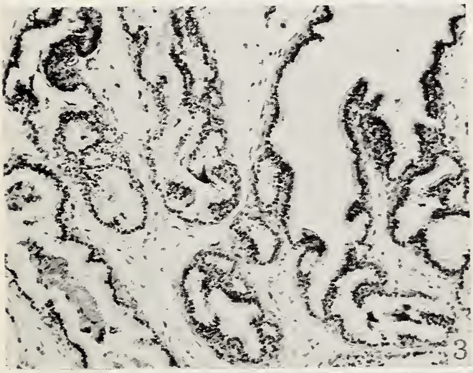
FIGURE 4.—Section through anterior prostate, from a 3-month-old mouse, cultivated for 10 days in air. *Note* reduction of epithelial projections. PAS-positive. × 115

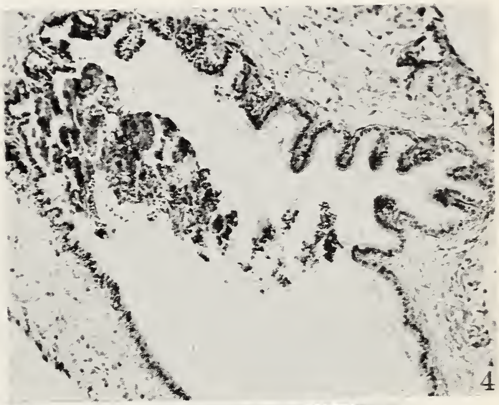
FIGURE 5.—Section through ventral prostate, from a 3-month-old mouse, cultivated for 10 days in an atmosphere of 95 percent O_2 and 5 percent CO_2. *Note* increased secretion as compared with explant kept in air. PAS-positive. × 115

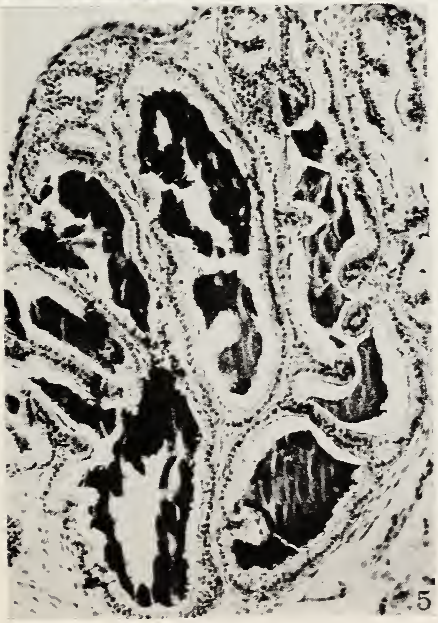
FIGURE 6.—Section through anterior prostate, from a 3-month-old mouse, grown in an atmosphere of 95 percent O_2 and 5 percent CO_2. *Note* close resemblance to organ *in vivo* (fig. 2).











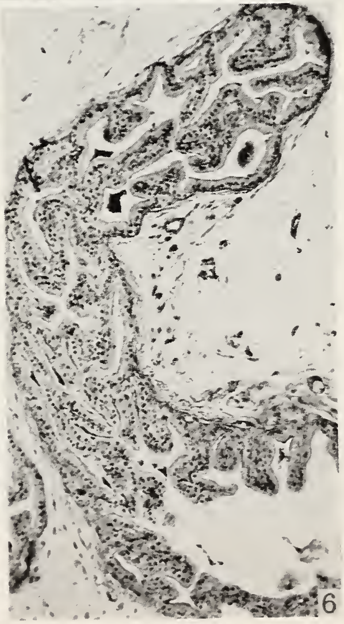


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PLATE 40

FIGURE 7.—Section through ventral prostate, from 4-week-old mouse, grown for 10 days in air. *Note* low, straight epithelium and increase of stroma. Hematoxylin and eosin. × 125

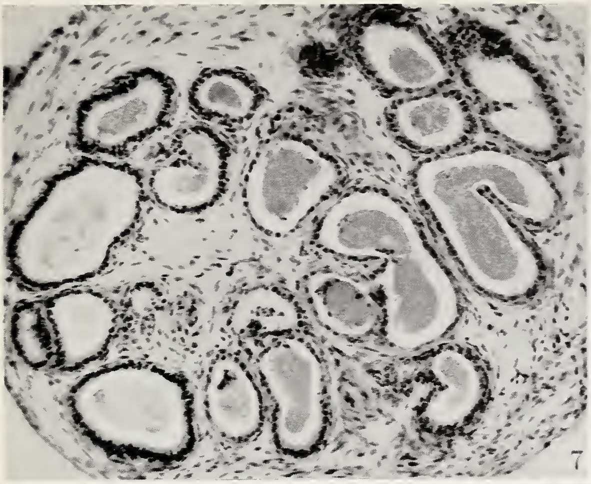
FIGURE 8.—Section through gland of same age grown for 10 days in presence of testosterone propionate. *Note* increased height and folding of epithelium and reduction of stroma as compared with figure 7. Hematoxylin and eosin. × 125

FIGURE 9.—Section through young gland grown for 3 weeks in presence of testosterone propionate. *Note* hyperplastic alveoli. Hematoxylin and eosin. × 90

FIGURE 10.—Section through prostate, from 5-month-old mouse, showing hyperplasia after 10 days' treatment with testosterone. Hematoxylin and eosin. × 115

FIGURE 11.—Section through prostate of 4-week-old mouse treated with estrone for 3 weeks. *Note* hyperplasia and squamous metaplasia of alveolar epithelium. PAS-positive. × 190

FIGURE 12.—Section through gland from 5-month-old mouse treated for 3 weeks with estrone, showing atrophy of epithelium and hypertrophy of stroma. PAS-positive. × 125



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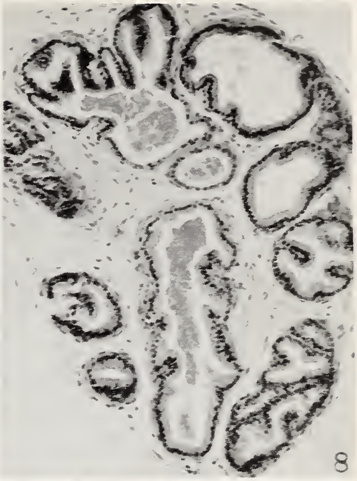

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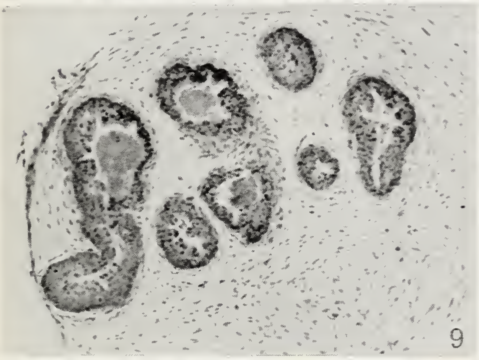

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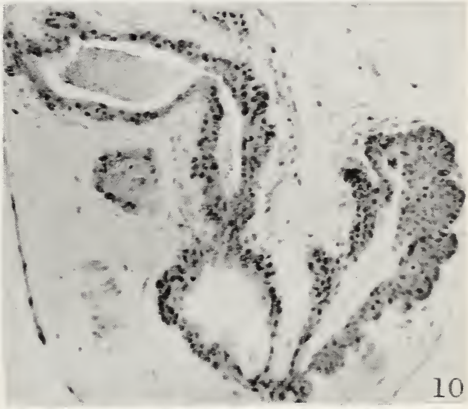

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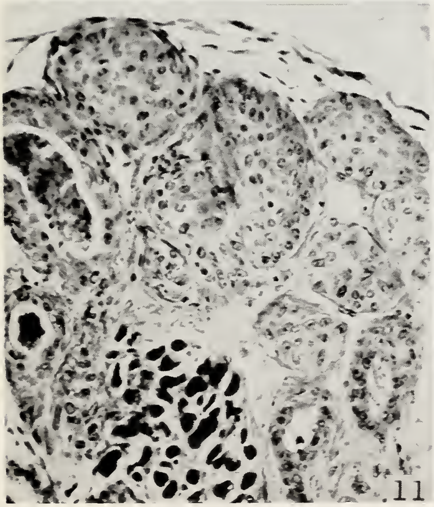

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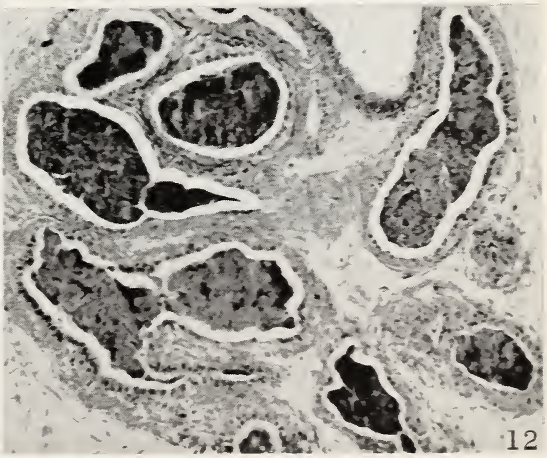

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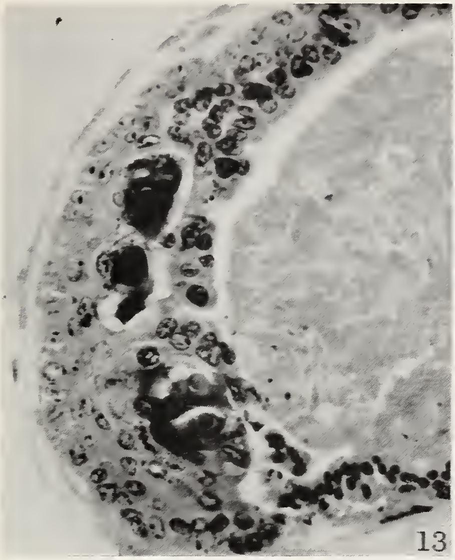
Plate 41

Figure 13.—Section through prostate grown for 10 days in defined medium. *Note* squamous foci beneath secretory lining epithelium beginning to break down. Hematoxylin and eosin. × 380

Figure 14.—Section through prostate grown for 10 days in defined medium, showing keratinizing and secretory epithelium in same alveolus. PAS-positive. × 230

Figure 15.—Similarly treated gland, showing alveolus in which the whole of the alveolar epithelium has undergone keratinization. PAS-positive. × 180

Figure 16.—Section through a similar gland grown in defined medium to which vitamin A had been added. PAS-positive. × 230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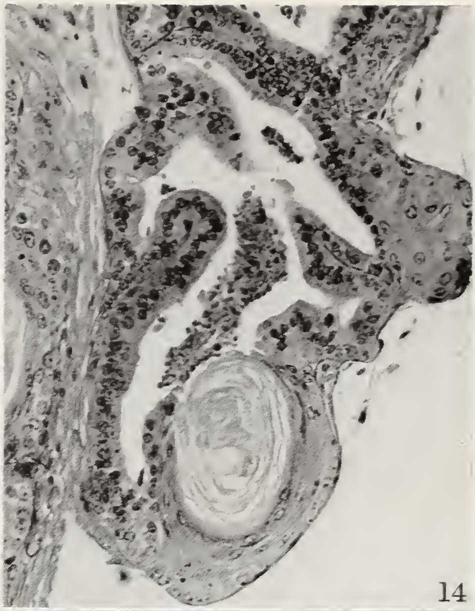
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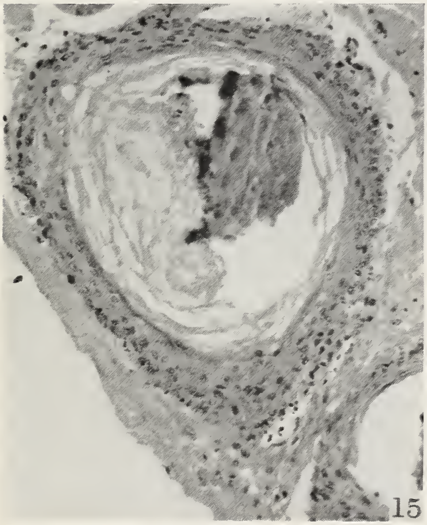
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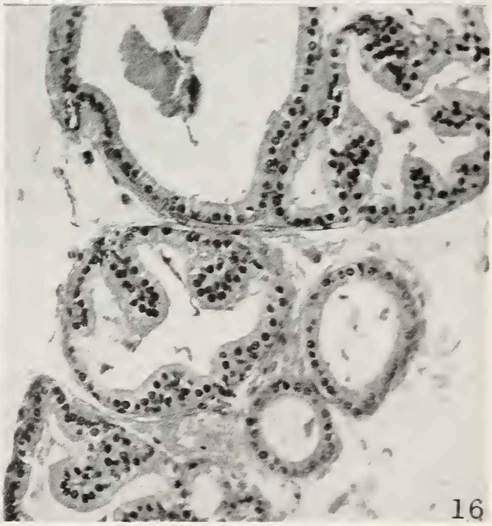
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PLATE 42

FIGURE 17.—Section through ventral prostate treated for 10 days with 4 μg per ml 3-methylcholanthrene showing reserve cell hyperplasia. PAS-positive. × 100

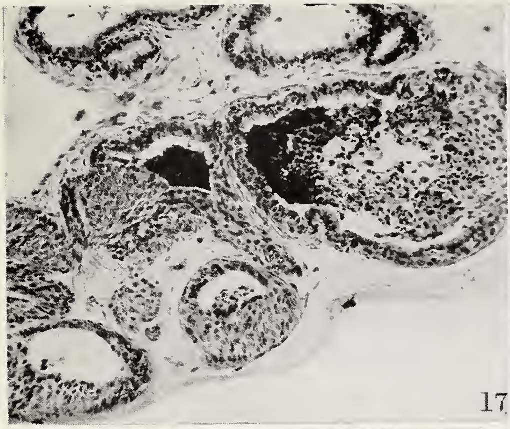
FIGURE 18.—Section through anterior prostate treated for 10 days with 4 μg per ml 3-methylcholanthrene. *Note* downgrowth of hyperplastic alveoli into the stroma. PAS-positive. × 90

FIGURE 19.—Alveoli in ventral prostate treated for 10 days with 3-methylcholanthrene, showing undifferentiated character of hyperplastic epithelium and the beginning of breakdown of secretory lining cells. PAS-positive. × 250

FIGURE 20.—Section through similarly treated gland, showing increased formation of secreting alveoli. PAS-positive. × 220

FIGURE 21.—Hyperplastic alveolus in anterior prostate grown for 10 days with 4 μg per ml 3-methylcholanthrene followed by 4 days in normal medium. *Note* increase in cell size. PAS-positive. × 250

FIGURE 22.—Section through similarly treated gland, showing loss of organization. *Note* irregular increase in size and multinucleate cells. Hematoxylin and eosin. × 400



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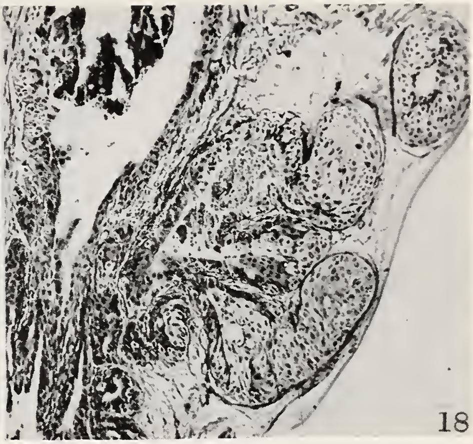

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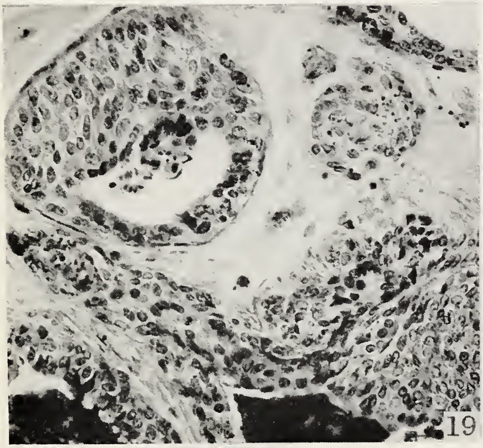

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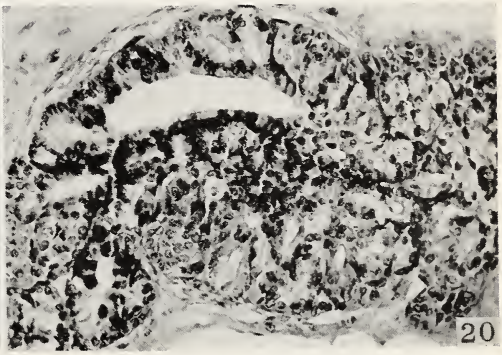

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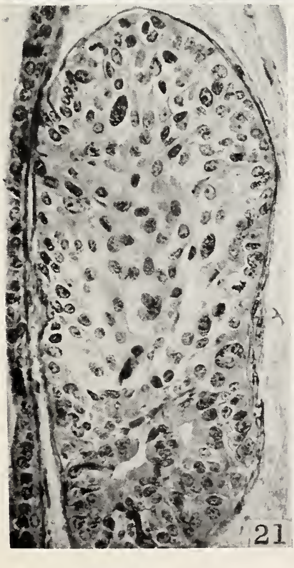

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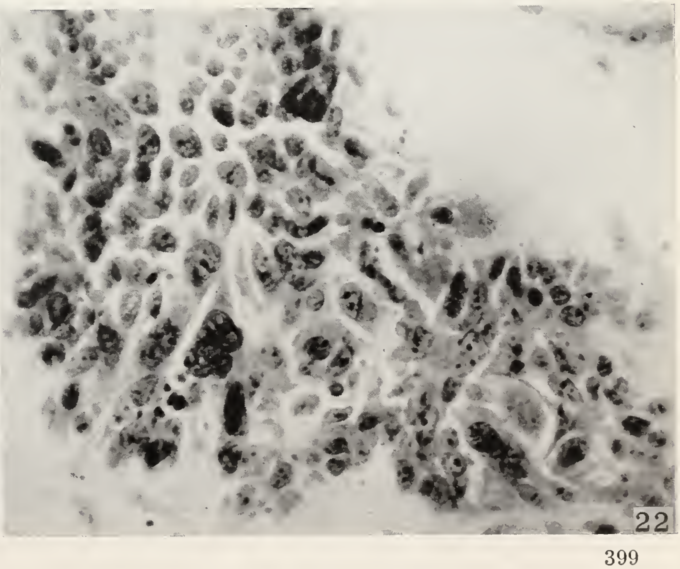

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Plate 43

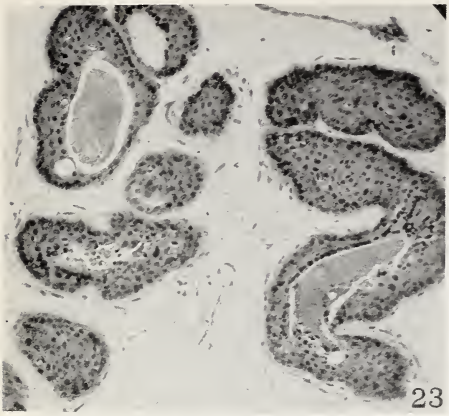
Figure 23.—Section through prostate grown for 10 days with 3-methylcholanthrene followed by 10 days in normal medium, showing loss of secretory epithelium and stratification of hyperplastic epithelium. Hematoxylin and eosin. × 115

Figure 24.—Hyperplastic alveolus in a similarly treated gland showing squamous metaplasia. Hematoxylin and eosin. × 285

Figure 25.—Ventral prostate gland treated with a combination of 3-methylcholanthrene and vitamin A, showing inhibition of squamous changes. Hematoxylin and eosin. × 120

Figure 26.—Alveolus in similarly treated gland, showing preservation of secretory lining epithelium and undifferentiated character of hyperplastic cells. Hematoxylin and eosin. × 560

Figure 27.—Prostate gland grown for 10 days with 3-methylcholanthrene, followed by cultivation in medium containing vitamin A. *Note* reduction of hyperplasia. Hematoxylin and eosin. × 120



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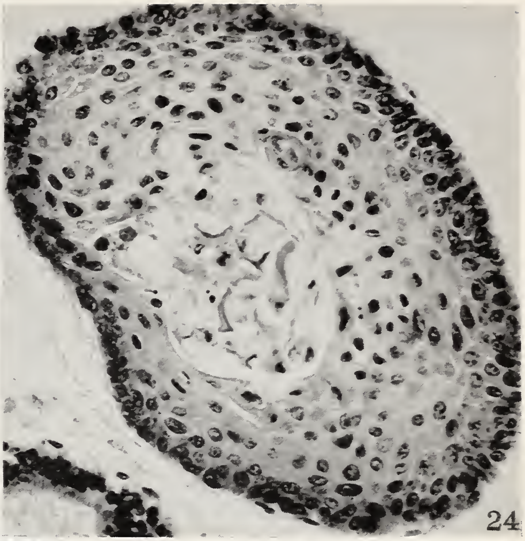

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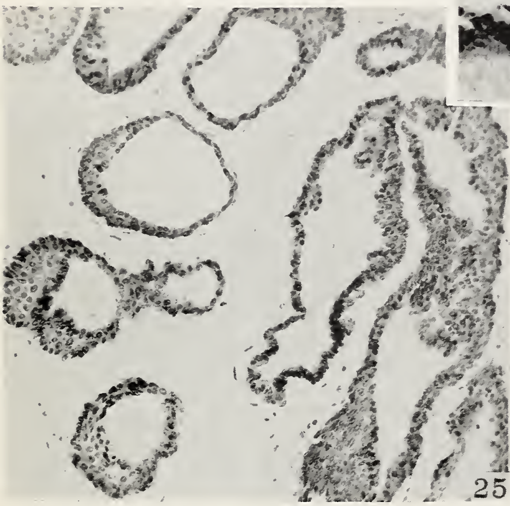

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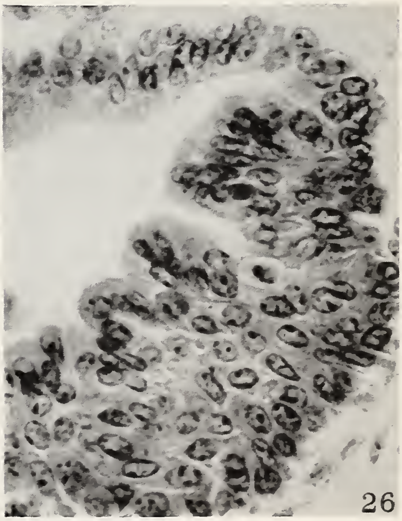

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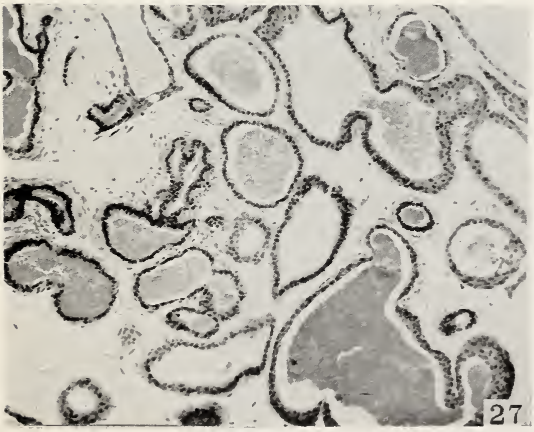

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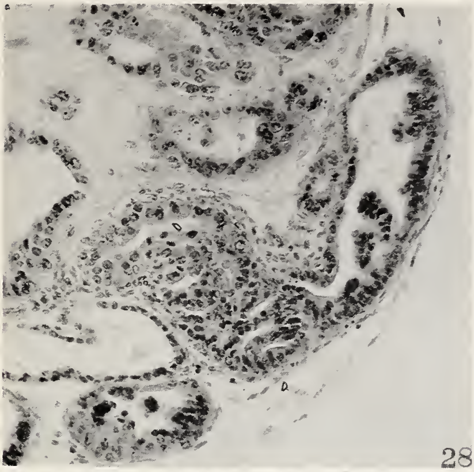
Plate 44

Figure 28.—Prostate gland from a 4-week-old mouse grown for 10 days with 2 μg per ml 3-methylcholanthrene, showing early hyperplasia. Hematoxylin and eosin. × 165

Figure 29.—Similar gland grown for 10 days with a combination of 3-methylcholanthrene and estrone, showing squamous metaplasia of hyperplastic epithelium. Hematoxylin and eosin. × 165

Figure 30.—Alveoli in 5-month gland grown for 10 days with a combination of 3-methylcholanthrene and estrone. *Note* atrophy of epithelium and increase of stroma. Hematoxylin and eosin. × 200

Figures 31 and 32.—Alveoli in young gland treated for 10 days with 2 μg per ml 3-methylcholanthrene followed by cultivation in medium containing estrone, showing degeneration of hyperplastic epithelium. Hematoxylin and eosin. × 200 and × 285



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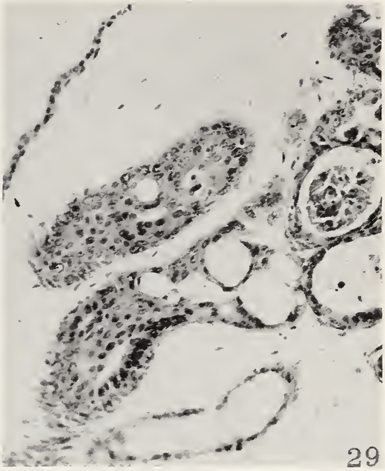

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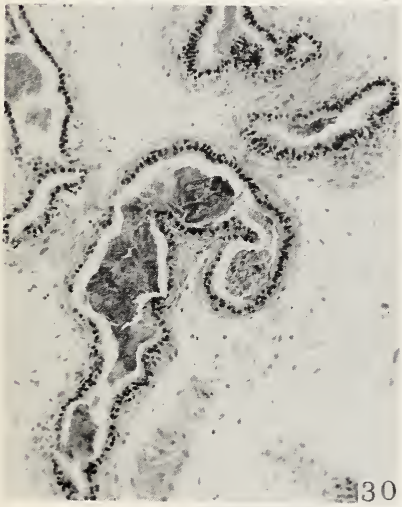

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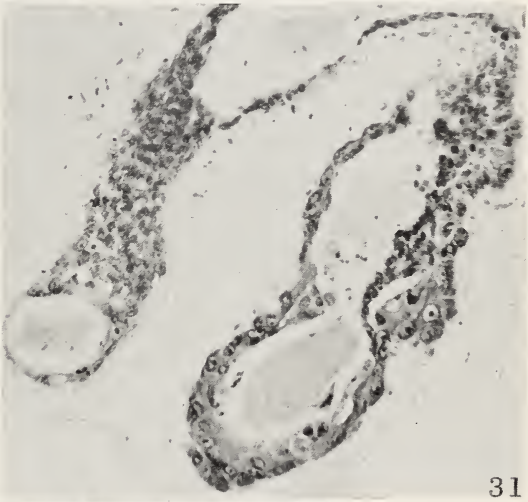

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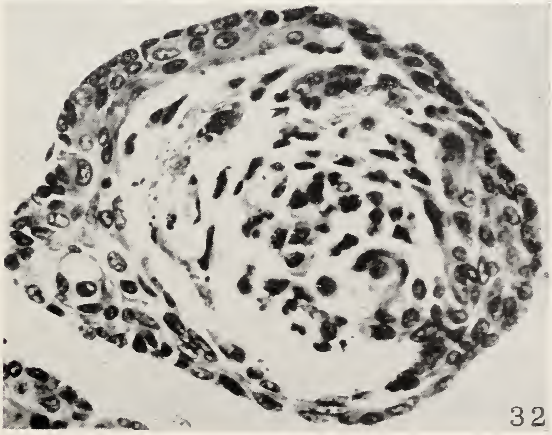

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DISCUSSION ON EXPERIMENTAL TUMORS; CRITERIA FOR DESIRABLE TUMOR SYSTEMS[1]

HORST SCHIRMER, The Johns Hopkins University, School of Medicine, Baltimore, Maryland

The criteria for induction of an experimental prostatic tumor in the animal may be listed as follows: first, the tumor has to arise within and from the prostate gland in the animal; second, it resembles the histological appearance of human prostatic carcinoma; third, it behaves much like human prostatic cancer in that it causes obstruction of either upper or lower urinary tract; fourth, metastasis takes place; fifth, in the experimental animal tissue transplantations may be performed; and sixth, the tumor should demonstrate some endocrine sensitivity. I congratulate Dr. Dunning and Dr. Fortner for having met most of these criteria.

To underline the various aspects of tumor induction, I should like to present some of our own experience with prostatic cancer. To us two factors seemed important: First, according to statements by Haddow and Horning, one should have atrophic glands; and second, derived from clinical pathological observations, prostatic cancer in the human arises mainly in the outer prostate, often associated with benign prostatic hyperplasia that compresses the normal prostatic tissue into an outer shell, which then harbors the foci of carcinoma. In the dog, ligation of the blood supply to one lobe of the prostate brought about a decrease in the size of one half of the prostate with atrophy of the prostatic acini. After the establishment of a possibly inactive and atrophic but normal glandular epithelium, a suspension of 3-methylcholanthrene crystals in normal saline was then injected into each lobe. This experiment resulted principally in acute and chronic inflammation, and in some instances abscess formation. Squamous metaplasia of the secretory epithelium could be observed in some glands. In a follow-up as long as 1.5 years we have found no cancer. Considering the dog's lifespan, one might have to allow for an extended latency in regard to cancer development.

The second experiment was conducted in the mature Wistar rat. Since Tannenbaum reported in 1943 that dietary measurements, specifically a high carbohydrate diet, can influence tumor induction as well as tumor growth, we have placed these animals into two major groups—one with normal diet and one with high carbohydrate diet. Each major group consisted of intact and castrated animals as well as rats receiving 25 mg of α-estradiol injected subcutaneously 3 times a week. The carcinogen was administered in the form of a silk suture soaked in 3-methylcholanthrene, dissolved in sesame oil, and driven through the ventral prostate in an S-shaped fashion. Starting with 150 g of total body weight, the animals on the high carbohydrate diet weighed 100 g more than those on the normal diet after 20 months. Thus far, 38 tumors have developed in 72 animals, with a latency range of from 6 to 12 months. Dietary and endocrine influence seem to be statistically insignificant. The tumors arose within and from prostatic tissue; they caused upper or lower urinary tract obstruction; they metastasized and could be transplanted; but they were not endocrine-sensitive. Histologically they were composed mostly of squamous cell carcinoma sometimes mixed with sarcomatous elements.

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

DISCUSSION ON EXPERIMENTAL TUMORS; OBSERVATIONS ON A SQUAMOUS CELL CARCINOMA [1, 2]

ALBERT SEGALOFF, Alton Ochsner Medical Foundation,
New Orleans, Louisiana

ALTHOUGH it was possible to convert a methylcholanthrene-induced tumor of the rat ventral prostate to an ascites form, we have abandoned this because keratin formation made transplantation and growth unpredictable.

After Dr. Huggins produced breast tumors by oral methylcholanthrene, we also attempted to induce adenocarcinoma of the rat prostate by the simultaneous administration of methylcholanthrene orally and testosterone propionate subcutaneously. These trials have been unsuccessful and have not produced such tumors.

Table 1 shows the growth characteristics in intact or castrated hosts of the strain of origin. These data clearly indicate hormonal sensitivity.

TABLE 1.—11095 Squamous cell carcinoma of the prostate.
Host: Fischer line 344

Induced: Methylcholanthrene crystals in ventral prostate	
Growth: young males:	9 days, 1.75 to 4.45 g
Adult males:	intact>castrate
Adult females:	castrate>intact
Young:	males>females

Surprisingly, as shown in table 2, though this tumor grows more rapidly in males than in females or castrated males, the administration of testosterone propionate significantly inhibits the growth of the tumor even in intact young males. 2α-Fluorodihydrotestosterone benzoate has a much lesser degree of inhibition, which may be due to its lesser androgenicity. A series of testololactones had no discernible effect on the growth of the tumor.

It is of further interest that castration after the tumor is established in males increases the growth rate, even though it grows more slowly if its entire period of growth is in castrated males.

Estradiol-17β by itself seems to have no effect on growth rate, but the

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1-3, 1962.

[2] This work was supported entirely by grant T-103-D from the American Cancer Society, Inc.

Table 2.—11095 Squamous cell carcinoma of the prostate.
Host: Young male Fischer rats

Hormone therapy	Mg per kg per day	% Control weight	Host weight change (g)
Testosterone propionate	50	77	+40
	100	36	+30
2α-Fluorodihydrotestosterone benzoate	50	91	+37
	100	68	+18
Various testololactones		No significant effect	

substitution of an 11β- or a 6α-hydroxy group produces growth-inhibiting steroids, as seen in table 3.

Table 3.—11095 Squamous cell carcinoma of the prostate.
Host: young male Fischer rats

Hormone therapy	Mg per kg per day	% Control weight	Host weight change (g)
Estradiol-17β	1. 0	99	+28
Estradiol-17β, 11β-hydroxy-	50	29	+1
	75	43	−4
Estradiol-17β-, 6α-hydroxy-, triacetate	50	38	−10
Estradiol-17β-, 6β-hydroxy-, triacetate	50	84	—

Corticoids significantly inhibit the growth of the tumor (table 4). In this experiment we have employed dexamethasone as a reference corticoid. The two 6α-substituted corticoids show substantial inhibition. It seems notable that 6α-methyl-11-ketoprogesterone also produces substantial growth inhibition.

These are only preliminary data, which are being presented in the hope that they will be of some help to this conference.

Table 4.—11095 Squamous cell carcinoma of the prostate.
Host: young male Fischer rats

Hormone therapy	Mg per kg per day	% Control weight	Host weight change (g)
Dexamethasone	0. 100	50	+7
	0. 316	50	+2
	1	17	+8
6α-Methylprednisolone	50	7	−18
6α-Fluoroprednisolone	5	57	+10
	10	12	−15
Progesterone, 11-keto- 6α-methyl-	50	33	−9
	75	9	−19

DISCUSSION ON EXPERIMENTAL TUMORS; THE IMMUNE STATE INVOLVED IN SUBCUTANEOUS IMPLANTS OF PROSTATIC TISSUE[1]

DAVID W. BISHOP, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland

In the few minutes available, I should like to discuss an issue which is, to our way of thinking, important in a broad spectrum approach to the biology of the prostate. Thus, if I seem to deviate from the subjects presented earlier, I may be excused, since biochemical components are involved and accessory gland tissue reactions are at stake.

Perhaps, as an oversight, the question of the immunology and/or the antigenicity of the prostate has been omitted from this program. I will not dwell on past attempts to inhibit or to counteract abnormal growth by administration of prostatic tissue or tissue components. Fifty-five years ago the outlook for this kind of therapy was brighter than it is today. While such considerations are yet current (*1–6*), the issue was pessimistically laid to rest, temporarily at least, during the recent symposium on "The Possible Role of Immunology in Cancer," particularly by the discussions of Southam (*7*) and Kidd (*8*). However, the application of another kind of immunologic tool with effects on the prostate has been presented during this symposium by Dr. Li in his discussion of anti-ICSH sera in inhibiting prostatic growth.

The immediate question of concern was touched upon by Geoffrey Bourne and Thaddeus Mann during these meetings and was implied years ago by the simple but elegant implantation experiments of Dorothy Price. These experiments clearly showed that accessory gland implants—essentially homografts into noninbred animals—can persist in subcutaneous sites. They undergo normal histological differentiation and retain biochemical function for periods up to 96 days—and probably longer. Seminal vesicular, coagulating gland, and ventral and dorsal prostatic tissue of the rat, mouse, and guinea pig can be successfully transplanted into unrelated recipient hosts; indeed, heterografts of accessory glands between different species will, on occasion, take. The latter type of graft acceptance was suggested at this Conference by Dr. Bourne, who described the implantation of human prostatic carcinoma into the brain of the rat.

In terms of current principles of skin and organ transplantation, these accessory gland implants are rather extraordinary. The usual skin

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[1] Presented at the Workshop on Biology of the Prostate and Related Tissues, Warrenton, Va., October 1–3, 1962.

homograft rejection reaction, essentially an immunologic phenomenon, occurs between about 10 and 20 days, and most tissue, organ, and parabiotic rejections between nonisogenic animals may be expected after similar intervals.

How is it possible that accessory gland tissue implants persist for such long periods? Of the two general immunologic explanations set forth to account for these phenomena, neither at present seems fully adequate. The suggestion that prostatic tissue, for example, is "nonantigenic" has little to support it; on the contrary, evidence by Flocks and co-workers (*3*, *4*) on human prostate and from our own laboratory on bovine prostatic tissue indicates that this is not so. Prostatic antigenic determinants evoke antibody production in the rabbit when tested either by the passive cutaneous anaphylatic reaction (*9*) or by gel-diffusion methods (*10*). The presence and relative significance of histocompatibility antigens in prostatic tissue have not, to my knowledge, been ascertained [*see* Ebert (*11*)]. The second postulate to account for the success of foreign accessory gland implants is that the grafts are somehow isolated from the immune defense mechanism. This is equally unlikely, since the implants are readily vascularized in sites that are immunologically responsive to the administration of a variety of antigens, soluble and otherwise.

We have initiated an investigation to determine what, if any, immunologic factors might be involved in the implantation of accessory gland tissues into subcutaneous sites. Three approaches have been undertaken, and the results thus far may be summarized as follows: 1) The sera of recipient rats, bearing viable implants of rat ventral prostate, fail to show detectable antibody when assayed by the passive cutaneous anaphylactic procedure; 2) the successful implantation of 8 to 10 prostatic implants fails to inhibit a second set of grafts from homologous donors; 3) rats, presensitized against ventral prostate administered intramuscularly in adjuvant, rejected homologous prostatic grafts implanted 4 to 5 weeks later. Thus, while the implants do not appear to sensitize the host sufficiently to evoke detectable circulating antibody or to impair the implantation of a second set of (homologous) grafts, the implants themselves are sensitive to the immunologic state of the host. Whether the immune response of the host is to be attributed to soluble or to cell-fixed antibodies has not yet been determined.

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EVALUATIONS

Chairman: Elwood V. Jensen

EVALUATIONS

Dr. Jensen: Assembled here are many of the world's experts on the various aspects of biology and chemistry of the prostate and related organs. We hope that you have been stimulated by hearing from each other on the status of current research. In this final session, we should like to hear your thoughts and comments about any phase of the whole subject, but in particular we invite your suggestions as to profitable lines of investigation that may facilitate the search for a complete solution to the clinical problem of prostatic cancer in man. If there ever was an opportunity to have someone tell us how to cure prostatic cancer, this should be it.

In this discussion, I think we should consider possibilities both of preventing prostatic cancer and of detecting it in its early stages so that the disease may be treated *in situ*. Regarding the major current problem of advanced metastatic cancer, we know that in many instances prostatic cancer, even though widespread, responds to antiandrogen therapy. Using our understanding of the biochemistry of the prostate, how can we predict which individuals will benefit from antiandrogen therapy and which will not? What can we do about those patients who do not respond, or who have become resistant to hormonal therapy? Finally, we have the condition of benign prostatic hypertrophy, perhaps not so dramatic a challenge as prostatic cancer but, because of its frequent occurrence, one that constitutes a major clinical problem. By better understanding of the basic biochemistry and physiology of the gland, will it be possible either to prevent or to ameliorate this condition?

Having explained what we would like to hear from you, I am going to call first on the members of this panel for their comments. Following that, the floor will be open for discussion.

Dr. Scott: Initially, I would urge further study of the nature of the lobes of the human prostate, in an effort to establish homologies, so that the information obtained in animal studies can be applied toward a better understanding of the human prostate and its disease processes. I have always been one to urge close cooperation between the basic scientist and the clinician. (In this regard, I can assure Dr. Lutwak-Mann that certain tissues can be provided if she wishes and that they could be sent by jet. It all depends on what kind of prostatic tissue is needed. Benign nodular hyperplastic tissue is plentiful, carcinoma less so, and fresh normal prostate is scarce.) There have been a number of examples of willingness to cooperate in research. For example, Dr. Dorfman, in an informal session, suggested that it would be important to measure testosterone levels in blood and urine in patients with prostatic cancer who were undergoing hormonal therapy. He not only suggested the idea but agreed to perform the analyses. We are also deeply indebted to Dr. Dunning for her discovery of an adenocarcinoma of the prostate of the rat. If this tumor proves to be hormone-dependent, it may be a very useful tool in screening compounds for use in the treatment of prostatic cancer.

As to the treatment of early prostatic cancer in humans, I would like to make a plea for early surgical excision, even though we do not know much about the potential for growth of this cancer, and even though the results are statistically not much better than with hormonal treatment. Concerning the treatment of disseminated prostatic cancer, I think that most agree that castration-estrogen treatment is the method of choice. It is not possible at this time to evaluate adrenalectomy or hypophysectomy, although according to my personal experience in 17 cases, hypophysectomy can at times be an effective treatment in the patient who has relapsed on castration-estrogen-cortisone therapy. A great deal more work must be done to determine the cause of prostatic cancer, whether it be hormonal, viral, or what.

Dr. Jensen raised the question of hormonal treatment and prevention of benign prostatic hyperplasia. This is extremely important, because even though the mortality incident to removal of a benign adenoma is only about 1 percent, prostatectomy is still associated with considerable morbidity. If it were possible to prevent nodular hyperplasia with a pill, to control prostatic cancer with a pill, and to increase libido and potentia at the same time, this would be wonderful. But perhaps it is not possible. Many attempts have been made to treat benign hyperplasia with androgens and estrogens, but none of these studies have been critical ones. As Clark of England pointed out years ago, a minimum period of evaluation of at least 44 months would be required to determine an effect. I know of no such study anywhere.

Dr. Pearson: I am certain that these basic studies will lead to new approaches to the human problem, which has been my area of interest in the past. The hormone dependence of prostatic cancer is a fact of clinical importance. Attempts to extend the palliative effects of orchiectomy and estrogen therapy need further review. The possibility that adrenal androgens were a factor in this disease led to study of adrenalectomy as a potential palliative procedure in patients who were in relapse after orchiectomy and estrogen therapy. Although some short-term beneficial effects were obtained, the results were not striking. Removal of the pituitary gland has also been done in a small series of cases and was based on the concept that pituitary hormones might have some synergistic effect with androgen on the growth of the prostate.

Dr. Scott mentioned his group of 17 cases in which some promising results appear. Dr. Bronson Ray, Dr. Willett Whitmore, and I have studied the effects of hypophysectomy in 15 patients with prostatic cancer in relapse after orchiectomy and estrogen therapy. Five patients had significant improvement after hypophysectomy. This approach has not been pursued with vigor, but I think it should be. The patients we studied in New York were essentially terminal. This was a correct approach in the days when hypophysectomy in man was a new and experimental procedure. Now, however, hypophysectomy is a definitive procedure, with little risk of morbidity or mortality, in the hands of an experienced neurosurgeon. I think that removal of the pituitary at earlier stages of prostatic cancer is one definite area to be explored and evaluated.

Dr. Furth: The proceedings of this Conference will be a record of splendid advances in knowledge of embryology, physiology, and biochemistry of the prostate. I have learned a lot. However, I should like to stress that there were no parallel advancements in the knowledge of the two major human diseases of this gland: benign hypertrophy and carcinoma. We are still ignorant as to what initiates these diseases and what makes them progress.

In the mechanism of carcinogenesis in general we distinguish two different types of agents, carcinogens and promoters. From recent observations we concluded that hormones are not carcinogens, but they are promoters in initiation and progression of tumor cells. So the question arises, what changes the normal prostatic epithelium into an autonomous epithelium? Two classes of carcinogens, chemicals and viruses, are likely possibilities. In experimental studies methylcholanthrene and other hydrocarbons were shown capable of producing squamous cell carcinoma of the prostate. Perhaps they can also produce adenocarcinomas under certain conditions, but they are not likely to be the natural carcinogens of the prostate of man. The presentation of Dr. Mann is of special interest in this connection. He has shown that a vast number of substances are either excreted or secreted by the prostate, which is both a holocrine and an exocrine gland. Some are stagnant and give rise to amylaceous bodies. Conceivably, some of the stagnating substances are carcinogenic. I know of no attempt to determine whether any of them have structural similarities to known carcinogens, and whether prostatic secretions or excretions are carcinogenic in animals. Let us remember that carcinogens are not site-specific. If applied topically, they can produce cancers at many sites. Carcinogen of the prostate, like that of cancer of the lung, can be tested on the skin.

I am more and more impressed by the importance of viruses in the genesis of many cancers. A steadily increasing number of cancers of animals are definitely linked to viruses. There have been great advances in methods of the identification of such viruses. Most of them are not species-specific or organ-specific. So far as I know, there has been no attempt to search for viruses in relation to prostatic tumors.

The prostate is an endocrine-dependent organ. Therefore, as concerns the genesis of prostatic tumors, let us consider the analogies of the thyroid and the mammary gland which are also hormone-dependent. In the genesis of the thyroid and mammary gland tumors homeostatic hormonal derangements are dominant. With respect to thyroid tumors there is usually some initial block in thyroid hormonal synthesis leading to stimulation of thyroid-stimulating hormone output. This is vital in thyroid tumorigenesis. In the genesis of mammary cancers, pituitary hormones, notably the so-called prolactin, play a significant role. (It seems to me that growth hormone and prolactin are Siamese twins. They may be different functions of the same molecule or are related molecules produced by the same pituitary cell called mammotrope.) In any case, hormones of the pituitary directly affect these glands. To my knowledge, a similar, direct connection between pituitary and prostate has not been established. Clarification of this situation is needed when we think of hypophysectomy or more generally of endocrine control of prostatic tumors. The hormonal influences mentioned at this Conference are nonspecific and common to most cells, *e.g.*, thyroxine and cortisone are needed in small amounts for growth and function of many cells. Gonadal hormones are specific stimulants or inhibitors of the prostate. Pituitary gonadotropes appear to act indirectly. Knowledge of the homeostatic regulation of prostatic growth and function, notably as related to tumorigenesis, is very meager.

The need for an experimental animal tumor similar to that of the human adenocarcinoma of the prostate has long been desired. An inroad has been made in this difficult area by Dr. Dunning, who discovered a transplantable adenocarcinoma of the rat prostate that probably will prove to be hormone responsive. It occurred spontaneously. Now that we know such a tumor can occur in the rat, I trust that others will be found and studied.

I should also like to mention the work of Dr. Lasnitzki that indicates how we can determine hormonal responsiveness of such tumors *in vitro*. Failure of cell cultures to respond to hormones was a disappointment for decades. It was discovered in recent years that organ cultures can respond to hormones, so there is now the possibility of determining the responsiveness of the given tumor to various hormones. This is well indicated by the work of Dr. Lasnitzki. At present, for lack of such a technique, therapy is too routinized. Tumors of an organ vary widely as concerns their responsiveness to hormones. Furthermore, they can change their responsiveness in the course of progressive growth. The original stimulant may become an inhibitor, and vice versa, as has been demonstrated with transplantable tumors. Therefore, a routinized therapy as is now carried out often does not work and sometimes may do harm. If a given prostatic cancer is sensitive to androgens, then antiandrogens are the logical therapeutic agents. If it is not sensitive, other agents should be looked for. Organ cultures have a potentiality as means of determining such sensitivities. Before the method is introduced into human practice, diverse experimental tumors with known hormone responsiveness *in vitro* should be tested in organ cultures, and thereby bring proof that the method works.

In conclusion, although knowledge in the genesis and control of carcinoma and benign hypertrophy of the human prostate is still very deficient, the technical advances we have learned about make us optimistic that the coming years will bring more rapid advances in these vital areas.

Dr. Brendler: One of the most valuable features of a conference like this is the little conferences which occur during the intermissions and between sessions. Dr. Scott alluded to the informal meeting at which it was agreed to collaborate in a very specific manner with some of our colleagues in the more fundamental disciplines. Dr. Vollmer asked me if I would relay some of this to you. One of the questions plaguing us over the years has been the relationship of the androgens, particularly testosterone, to clinical prostatic cancer. We have been searching diligently if not effectively for antiandrogens, and we wonder just how important androgens are in prostatic cancer growth. Twelve of us met to discuss this point, and the arguments against testosterone as a factor in human prostatic cancer were summarized as follows by Dr. Vincent Hollander: 1) It is well known that administered testosterone does not exacerbate prostatic cancer in most patients. In fact, in the great majority of patients, testosterone has no effect whatsoever. 2) The administration of testosterone actually improves a small number of these patients and in some cases this improvement is very dramatic. 3) Testosterone assays of spermatic vein blood are low to normal in patients with prostatic cancer.

Although the foregoing 3 points have to be qualified, they do show the necessity for studies of testosterone levels in blood and urine. Dr. Dorfman has kindly consented to work with us in this study. We are confining our study to patients with active prostatic cancer with metastases and with elevated serum acid phosphatase levels, if possible, who have never before been treated. Our reason for this is that clinical experience has shown us over the years, and more recently following studies sponsored by the Cancer Chemotherapy National Service Center, that patients who have relapsed after castration are probably hormonally insensitive. They will no longer respond to diethylstilbestrol, testosterone, or adrenalectomy. There are exceptions to this, but in general these patients can be regarded as hormonally insensitive. We therefore plan to focus on patients with active disease and who have not had prior therapy.

I should like to comment on experimental tumors. We must be very careful to try to develop a tumor which fulfills the criteria enunciated by Dr. Schirmer. Every effort must be made to assure ourselves that such an experimental tumor model reproduces its human counterpart more or less faithfully. If we do not, the result may be a tumor that will merely serve to screen steroids or other agents, but which will not provide a suitable experimental model of human prostatic cancer. This is not meant to belittle Dr. Segaloff's results, which have impressed me very much, but I do believe we must bear this point in mind.

Dr. Babcock: As a misplaced chemist on this panel I would like to echo Dr. Scott's comments that we have seen much cooperation among disciplines, without which we could not expect the progress that has been made. Although this workshop has come up with a number of solutions to problems of prostatic cancer, it has inevitably raised more questions than it has answered.

We learned from the pathologists and electron microscopists that enzymes, some of which may be concerned with steroid transformation, change in concentration and location under the influence of testosterone and other hormones. Dr. Tagnon has described a property of acid phosphatase that distinguishes the cancerous prostate from normal prostate, namely, the inactivating effect of the calcium ion. Further studies of this type should turn out to be extremely profitable.

It is well known that the ovary secretes not just a single hormone but a variety of hormones based on oxidative, enzymatic transformations of progesterone: progesterone itself, 16-hydroxyprogesterone, testosterone, estradiol, and others. The adrenal gland also secretes a wide variety of related hormones. It seems strange that our knowledge of testicular secretion is limited to a few hormones only. Are there other substances that the testes produce, which on their removal are also eliminated?

The work described by Dr. Saunders, relating structural changes at C-3, C-17, and C-19 of a cross section of steroids with specific effects on the prostate and seminal vesicle, brings to mind similar data from the Cancer Chemotherapy National Service Center bioassay program. Thus the orally potent anabolic agent Halotestin is unusually free of prostate-stimulating activity, though fully effective as an androgenic and myotropic agent. The 9-fluoro-11-hydroxy group has thus markedly changed the activity seen with methyltestosterone and testosterone. Related compounds with the fluorine now at C-2, 2-fluorotestosterone and 2-fluoro-17-methyltestosterone, appeared to behave in quite the opposite manner, being highly selective stimulators of prostatic growth. The wide selection of steroids now available in the Cancer Chemotherapy National Service Center program, screened in suitably predictive systems, clearly will provide new and promising agents for clinical trial.

Finally, Dr. Pincus mentioned something which I think is very important and often overlooked. He referred to the possible stimulating role of progesterone on the prostate. This steroid is a precursor of testosterone which in turn is a precursor of estradiol. Why is it that after 40 the testosterone output in man and the estrogen output in woman decrease markedly, even though gonadotropin output appears to be elevated? Since the output of these steroids decreases with aging, why is it at *this* time, rather than when testicular output is at a maximum, that benign metastatic hyperplasia and prostatic cancer more often become clinical problems?

I feel that greater emphasis might be placed on the effect of hormones related to progesterone on the prostate. The study of progesterone suffers from the problem that exogenously administered material is extremely short lived. Today we have more potent agents suitably protected from enzymatic inactivation, such as Provera and many others, which are modified both in the A–B ring and in the side chain, protecting these key positions from reduction. I believe this class of steroids will prove to be of interest for further study. Similarly, the 11-oxygenated progesterones, mentioned by Dr. Segaloff, which appear to be effective antitumor agents in the Cancer Chemotherapy National Service Center's tumor tests and to inhibit prostatic growth, should offer hope for further progress in this difficult area.

Dr. Leathem: Biologically, I have always been impressed with the question of prostatic cancer, particularly as limited to man and dog, and whether it was not possible with further biological survey to find other species in which these tumors would be found. It seems from this discussion that prostatic cancers may be present in other species and that to the biologist this is the long-needed tool.

It is curious also that experimentation, as generally devised in the laboratory, does not greatly concern itself with the truly older animal and its capacity to respond to steroids. I was impressed by the statement that we are dealing with 250-day-old animals. It seems more likely we should be dealing with animals 600 or 700 days old, and should compare their capacity to respond to hormones with very young animals rather than those which are intermediate in their reproductive life. Dr. Brendler made a comment in his presentation about the dog, and whether it does or does not respond to therapy. We have had in our laboratory the opportunity to have dogs referred to us with many different cancers, including prostatic cancer. In two instances the dogs were treated: One was given estradiol benzoate, the other diethylstilbestrol, and the basic result was death. The difficulty with the whole experiment is rather typical—when these animals were referred to us, an initial biopsy was not obtained. The animal was autopsied and in one instance there was a carcinoma. However, another biological question arises. The dog is extremely sensitive to estrogen, and many years ago it was shown that diethylstilbestrol in doses of 2 or 3 mg per day will kill virtually any dog in 30 or 40 days. What happens to these animals, however, when they have a tumor? Whether it be in the reproductive system or not, are they more sensitive or less sensitive to the drug offered to them? I feel that considerably more investigation is necessary, on a broad biological base.

Dr. Mann's investigations on broad biochemical measures are beginning to give us clues to species differences which, I think, is an area we should foster, and attempt to get not only species differences but age differences in responsiveness. As to the question of tissue growth, we had some excellent contributions from Dr. Kochakian and Dr. Williams-Ashman on how these tissues respond and how they synthesize protein. However, we need a great deal more information about this particular functional transformation that is occurring in abnormal tissues. Transplantable tumor, we think, is perhaps tissue that acts as a nitrogen trap. Does the prostatic cancer act in the same way as a transplantable tumor? For this there may be an answer. I would appreciate having it. These particular thoughts occur to me because the effects of the over-all animal nutrition and nutritional status also raise the question as to whether we can provide what we sometimes like to talk about as a "prescription diet" to be associated with the drug therapy as part of a specific treatment.

Dr. Hollander: I would like to discuss: 1) the role of testosterone in prostatic cancer, 2) the role of pituitary in prostatic tissue, and 3) the meaning of hormonal sensitivity.

Current concepts of the regression of prostate cancer after orchiectomy involve a testosterone requirement by the tumor epithelium. It is important to remember that this concept is a reasonable assumption and not a fact. Dr. Brendler has put forward the arguments that we have discussed and I think they are quite compelling. It would seem worthwhile to evaluate carefully the underlying assumptions regarding the mechanism of tumor regression following orchiectomy. In our laboratory we have studied the testosterone level of spermatic vein blood and find that it is a function of age. It decreases sharply in older men and no difference was seen in spermatic vein blood testosterone level from individuals with prostate cancer and those who so far as we knew did not have this disease. Many of the levels seen in older individuals were very low indeed. Further study of the spermatic vein blood might be very rewarding in this regard. Prelog examined the steroids present in testes and was able to isolate an impressive number of substances. Techniques are available for examining spermatic vein blood by the use of the isotope derivative technique, countercurrent separation, gas chromatography, etc., which would permit the detection of very small amounts of steroid hormones and their separation. This might lead to the detection of other substances that changed with age and which might be more important for the stimulation of this tumor.

However, the possibility that the substance in the testicle which stimulates the growth of prostate cancer may be water-soluble should not be overlooked. The testicle under very abnormal circumstances can produce a substance which physiologically acts like pituitary hormone. These effects are particularly evident in choriocarcinoma. It is possible that such substances can be produced by the normal testicle but has so far escaped detection. Possibly the production of such a trophic substance would actually increase in the older person due to decreased steroid feedback. The role of the pituitary as discussed by Grayhack and Li opens up the possibility, discussed by Dr. Pearson, of early hypophysectomy. I would like to second this suggestion. I am convinced that hypophysectomy should not be abandoned in the treatment of prostate cancer until it is evaluated at a much earlier period in the disease. I understand the reluctance of physicians to advise an apparently radical procedure when the administration of estrogen or simple orchiectomy is so likely to be effective. However, we are quite certain that the latter procedures never result in cure, and hypophysectomy is no longer considered as such a dangerous or radical operation.

The meaning of hormone responsiveness of tumors and of their eventual autonomy is under active investigation in several laboratories at the enzymatic level. What is the mechanism by which a tumor escapes control? It seems to me that there are two major possibilities: 1) The tumor cells have lost the enzyme system responsible for the control mechanism. This deletion hypothesis has much to recommend it. It is known that in some tumors gradual progression involves loss of enzymes and the tumors become more and more like all other rapidly growing tumors. 2) That the tumor may become oversensitive to the growth-promoting substance in question has not been adequately explored. If this occurred the tumor might actually appear nonresponsive, because ablation of the pertinent organ producing the growth factor might not result in tumor regression. The tumor might not regress because other sources of the growth substance produce enough stimulating substance under these conditions so that the tumor growth continues. If the tumor were very responsive indeed, then it would not respond by increased growth to the administration of this stimulating substance because it was already maximally stimulated. I have no evidence supporting this concept, but it is a logical possibility. The deletion hypothesis is an extremely ominous concept, because it is difficult to see how we could reintroduce the controlling enzyme system that was deleted.

Dr. Segaloff: Throughout this Conference I have wondered why the lion is omitted from the animals who develop spontaneous cancer of the prostate.

I am delighted that Dr. Hollander did not talk about hormone dependence. Hormone sensitivity or responsiveness would seem better terminology in the light of what we have learned here.

Apparently we can benefit in our studies from tumors which do not fulfill all the criteria mentioned by Dr. Brendler. We now have a series of biologic tools, and we will have more as we develop them, to assist in the selection of agents to try in our final court—*i.e.*, men with cancer of the prostate. We had hoped that the growth of human tumors in animals and tissue cultures would solve the problems of finding effective agents against these human tumors. We now have human tumors growing in this manner, but as yet they have brought no striking new answers.

I am impressed that hormonal therapy, beyond the first almost uniformly successful castration or estrogen administration, is seldom of assistance. We should therefore consider an attack on this tumor with cytotoxic agents. Since cancer of the prostate characteristically grows in and destroys bone marrow, we must bolster the marrow to permit administration of adequate amounts of cytotoxic agents.

Finally I would like to ask whether the prostate synthesizes protein out of proportion to its increase of protoplasm or is this a clue to the actual site of action of androgens?

Dr. Williams-Ashman: I regret that I have nothing to say about prostatic cancer. Indeed, I think it is rather depressing that so many of the basic studies, on the chemical physiology of male accessory glands, carried out over the last two decades have had comparatively little application to the understanding or treatment of neoplastic diseases of the human prostate. However, investigations of the biochemistry of the secretions of these organs, in which Dr. Huggins and Dr. Mann have pioneered so greatly, have had fruitful application to problems of fertility, over and above their intrinsic scientific interest. But one cannot overemphasize the paucity of our knowledge of normal prostatic physiology, especially of secretory mechanisms in the prostate gland. In particular, I would like to underscore Dr. Mann's and Dr. Lutwak-Mann's remarks on how little we know about the permeability of prostatic epithelium to various substances. We do not know how many of the substances in normal prostatic fluid are actively secreted or are simply washed out during the secretory process. Only a few sporadic observations have been recorded concerning the passage of foreign substances into prostatic secretion. Again, practically nothing is known about the normal rates of secretion of prostatic and vesicular fluids in man. I wonder to what extent, if any, secretions stored in the lumina of the prostate gland and seminal vesicles can be reabsorbed into the circulation. The whole question of the role of stasis of prostatic secretion in human prostatic disease certainly merits more investigation.

The most fundamental problem in prostatic physiology is undoubtedly to define, in precise physicochemical terms, the molecular mechanism(s) which are responsible for the action of sex hormones in controlling the functional differentiation of this organ. It is unlikely that this problem will be solved by a single experiment. Up to now, experimental approaches to the problem of sex hormone action have been almost entirely phenomenological in nature. There has been considerable stress on measurements of the activity of various enzymes, or multienzyme systems, in the prostate gland in relation to administration or deprivation of gonadal hormones. However, although such studies have disclosed many large and reproducible changes in enzymatic activities accompanying the actions of sex hormones on the prostate, it would seem likely that most of these enzymatic changes are secondary to more fundamental processes which are set in motion by steroids. Again, unequivocal evidence has been obtained over the last decade that certain sex hormones can participate directly, and in a catalytic manner, in the action of certain hydrogen-transporting enzyme systems in the test tube, but there is no compelling reason to believe that such reactions are the chemical basis of sex hormone action.

There is a growing realization in some quarters that the fundamental locus of action of sex hormones may lie not in an influence of any of the enzymic reactions normally involved in intermediary metabolism of fats, proteins, or carbohydrates, but rather in the chemical mechanisms involved in expression of genetic information. Perhaps the problem will resolve itself into an experimental analysis of factors which determine the expression of those parts of the genome (encoded in the deoxyribonucleic acid) that harbor information for the synthesis of proteins controlling the androgen-dependent functional activities of the prostate gland. In reply to Dr. Segaloff's question, it will obviously be necessary to determine the nature of the proteins synthesized by cell-free preparations isolated from the prostate gland.

* * *

Dr. Jensen: This concludes the formal discussion. Now I should like to invite comments from all of you. To begin, I think it appropriate to ask the two principal speakers, Dr. Price and Dr. Mann, to express their opinions.

Dr. Price: I should say a word about lions since Dr. Segaloff has brought up the question. I have thought, too, that lions might be a good possibility for study. They are popular animals in zoos and are probably kept to advanced ages. I think that Dr. Huggins used to say that benign hyperplasia of the prostate occurs only in man, dog, and lion. However, I have not been able to find any record, up to the present time, that lions are troubled that way. Incidentally, the prostate gland of lions does not completely surround the urethra as it does in dogs.

I would like to comment on the need for approaching some of the problems of prostatic pathology in a very broad biological way. I am much impressed with the importance of age in the development of benign hyperplasia and prostatic cancer. The prostate undergoing normal senile involution in man is resident in an aging body in which all tissues and organs are undergoing various aging processes. The endocrine glands are certainly doing so and the hormone milieu of the prostate gland is changing. One example is the changing androgen/estrogen ratio. It seems to me that in order to try to understand the susceptibility of the aging prostate to pathology, we should find out more about the prostate itself, and much more about changes produced by aging in structure and function in all glands of the endocrine system. This need has long been recognized, but it is a good idea to think more about it.

Dr. Mann: It is obvious that there is no scarcity of problems still awaiting solution in the field of physiology and pathology of the prostate. The difficulty is to decide where to begin and in which order to tackle the many questions.

As to biochemistry of the prostate, it must be remembered that this is a relatively new area of investigation, hitherto dealing mainly with the normal gland. The chemical pathology of the prostate in its manifold aspects, some of them touched on during our sessions, still remains to be explored. Three approaches of biochemical experimentation capable of profitable development are those linked with hormonal, enzymic, and secretory mechanisms.

As regards the response of the prostate to hormonal stimuli, much remains to be learned about the mechanism underlying the action of testosterone and other steroids on the normal prostate gland. We might then perhaps be able to detect distinct differences in the androgen metabolism between a normal, a hypertrophic but nonmalignant, and a cancerous prostate gland.

The present state of enzymological studies on the prostate leaves much to be desired. Numerous claims have been advanced in the past with respect to alleged increases or decreases in enzyme "content" or "concentration" after various treatments, but on closer inspection of the techniques employed one finds that what has been measured in most instances was total enzymic activity, and not the true content or concentration of an enzyme. Too little attention has often been paid to the fact that an enzymic activity, particularly when studied in whole tissue, is the outcome of an intricate balance between numerous interacting factors which, in addition to the enzyme itself, include various coenzymes, activators, inhibitors, and the state of cell permeability, that is, the degree to which an extracellular substrate can be made available to an enzyme located within a cellular structural element. The existing confusion is clearly due to misunderstanding of fundamental principles underlying enzyme-substrate interactions.

There is now available an ever lengthening list of substances known to be secreted by the normal prostate gland; some of them, for example, acid phosphatase and citric acid, have also been studied in the hypertrophic and cancerous gland. But there is too little information as yet about differences which may exist in the normal and abnormal gland, and the chemical nature of the secretory products produced by the normal and abnormal prostate, during metabolism of lipide, protein, carbohydrate, nucleic acid, etc. There appears to be also a pressing need for further ultrastructural studies on the elements responsible for the secretory processes in the epithelium of the normal and the pathologically altered prostate gland.

Dr. Bern: Coming, as does Dr. Laznitski, from a laboratory where organ culture is a part of the daily ration, I would like to emphasize something that Dr. Furth raised about the possible applicability of this technique. Here is a field where fundamental biologists have been working for some time. It does seem to me that with a relatively small amount of effort the methodology of organ culture might be used specifically in working with human material from the clinic. I can think of two major areas wherein it might be quite helpful: One has to do with the desirability of characterizing the hormone responsiveness and sensitivity of tumors, from individual patients, by the use of biopsy samples. An organ culture methodology could become auxiliary to the ordinary histopathologic examination. Dr. Segaloff's point about wanting to know something about hormone dependency—though he does not like that term—can be investigated in an absolutely hormone-free medium through the use of organ culture techniques. Humoral factors that might be associated with aging could also conceivably be explored by these techniques. The second major area—one that I have discussed with Dr. Taylor—concerns the chemotherapy screening program and the possibility of using human tumor samples in organized screening efforts, thus to acquire information directly applicable to the prostate problem in man. One may not have to depend on finding a rat tumor that is like the human tumor and which in the course of serial transplantation may lose the very characteristics that made it initially of interest.

Dr. Pearlman: I believe that a comparative study of target sexual tissues, such as the ventral prostate, breast, and uterus, under hormonal stimulation might be profitable. It seems to me that these tissues may share to a greater or lesser degree certain biochemical mechanisms that underlie the biological response to hormonal stimulation. This, of course, is not a novel or original thought, and I would like simply to make the suggestion that a workshop, similar to the one we are about to conclude, be devoted to comparative aspects of target sexual tissue response. Such a comparative study would draw from many scientific disciplines, *e.g.*, anatomy, biochemistry, and pathology.

Dr. Farnsworth: One of the problems that the biochemist is faced with is the unavailability of normal human prostate tissues. We are always tempted to go to the autopsy table but we are reserved about doing this, since we wonder whether our results would be valid. Can anybody relate to me any experience in this direction? How can we get to study the normal human prostate gland?

Dr. Scott: How much tissue and how fresh?

Dr. Farnsworth: To do a biochemical study one could do some preliminary work with a gram, but 5 grams of tissue would be more useful.

Dr. Scott: If you want 5 grams of tissue, the only way that I know how to get this much would be surgically, for example, at total cystectomy for bladder cancer, in which operation the prostate and seminal vesicles are removed as well. However, the operative procedure itself, which requires several hours, might result in profound interference with the blood supply to the prostate, and this tissue might end up no fresher than that obtained at autopsy. Smaller amounts of tissue can be obtained with a small trocar or needle used for biopsy. This might provide enough tissue for cerain electron microscopy studies as well as histochemistry.

Dr. Lasnitzki: We found that 100 milligrams of prostatic tissue were sufficient for biochemical studies, *e.g.*, for measurements of amino acid utilization, arginase activity, and oxygen uptake.

Dr. Bourne: We have heard many excellent papers concerned with the biochemistry and physiology of the prostate. Many refer to the weight of the prostate in assessing the significance of these studies. Both Dr. Segaloff and Dr. Mann have put their fingers on a key aspect of this problem, by referring to the possibility that the prostate can increase in weight by hydration. I am not clear from most of the papers whether the figures for enzyme activity in the gland refer to wet weight or dry weight of the gland. The fact that the gland can become hydrated as a result of experimental treatment can lead to very deceptive results if they are not referred to dry weight. Another possible source of error is the composite nature of the gland itself and the fact that this composition is not a stable one. For example, there is a glandular portion, and a stroma which is itself composed of several elements including smooth muscle and collagen. Analyses of prostate are an average of these tissues, and if one element decreases, the analysis will be dominated by those elements which do not decrease. A similar situation existed in earlier work on muscular dystrophy in which extensive increase in the connective tissue of the muscle in the course of the disease completely disoriented the analyses of the muscle, since all the biochemical changes were treated as changes in the muscle fibers. This was finally corrected by referring all biochemical analyses to non-collagen-nitrogen.

In the prostate after castration there is progressive decrease of size of acinar cells but at the same time there is a progressive multiplication of the smooth muscle cells. Biochemical changes represent an average between these processes. When compounds increase the size of the prostate, this can be due to a number of reasons: 1) water, 2) connective tissue stroma, 3) smooth muscle, or both stroma and muscle, and 4) increase in number of acini or increase in size of acini. Increase in size of acinar cells may be due to increase in water or protein. The gland may also increase in weight because of increased production of secretion. I should like to make a plea that all these weight-increase studies at least be correlated with a simple histological examination. Preferably, physiological and biochemical studies should be correlated with histological, histochemical, and fine structural changes. I do not believe we will really understand the mechanisms in the prostate until some group using these techniques applies itself to the study of the normal and abnormal prostates and their physiological responses.

Dr. Brandes: I would like to comment on the acid phosphatase that is so widely used to analyze the response in the patient. Through the work of De Duve it is known that there are sacs surrounded by very firm membrane and that it requires a very severe treatment, like sonication or treatment with detergents or enzymes, to release the action of the enzyme. From some of our studies we now conclude that in the prostate two types of acid phosphatases occur: One is present in secretory granules, and one is in close relationship to the lysosomes. The one in the secretory granules may be largely affected by castration or estrogen therapy which seems to produce a decrease in secretory gland activity, but the lysosome enzyme should also be considered. This also might be related to the finding of Dr. Tagnon with respect to the effect of calcium ions on the activity of acid phosphatase. It is possible that calcium may influence the permeability of the lysosome membrane.

Dr. Lutwak-Mann: I have no comment to make but would like to ask a question of the clinical experts. Is it at all conceivable that the human or the dog prostate could be influenced by substances present in the urine, especially after the permeability of the urethra has been altered by aging?

Dr. Scott: I am not sure I can answer but this much seems clear: When one makes a cystourethrogram by injecting radio-opaque solutions up the urethra, one rarely observes reflux of dye up the prostatic ducts. Hence, the urine probably does not normally come into contact with the epithelium of the prostatic ducts.

Dr. Mann: Even when an enzyme has been purified, it is not always possible to define its activity in terms of the absolute content of a single protein. According to some recent observations by E. J. King and his associates, the activity of the human prostatic phosphatase resides in more than ten electrophoretically distinguishable protein components; there is no reason to assume *a priori* that each of these is endowed with the same activity.

Dr. Tagnon: I should like to add a comment in favor of the view that it is preferable to talk in terms of enzyme activity rather than amounts. What we measure in biological material is activity, and we do not know until a final and complete analysis of all factors has been performed how this is related to the amount of enzyme present.

In this respect, we should remember that the prostatic secretion contains a proteolytic enzyme active at *p*H 7 to 7.4 and is present in all crude extracts prepared from the human prostate. It is probable that certain enzymes in these extracts represent adequate protein substrates for this proteolytic enzyme. Unless care is taken to prevent proteolytic action during the preparation and assay of prostatic enzymes, it may very well be that measurements are vitiated by the destruction of certain enzymes through proteolytic action. Therefore past studies on the human prostate, in which this source of error has not been considered, are perhaps subject to revision. Concerning the prostatic acid phosphatase, it would seem that this is not a substrate for the proteolytic enzyme, which therefore does not influence the level of acid phosphatase.

There are excellent inhibitors of the prostatic proteolytic enzyme, among which the following are particularly active: 1) the trypsin inhibitor from soy bean flour and 2) ϵ-aminocaproic acid.

Another factor that may alter the activity of enzymes in prostatic extracts was illustrated in our discussion: The presence of calcium ions may modify the acid phosphatase activity without modifying the amount present. Therefore quantitative measurements of prostatic acid phosphatase in body fluids should always include an adequate control of this calcium effect. Here again, it is possible that some of the past work done on acid phosphatase may need revision.

In answer to some of the other questions, the calcium effect on prostatic acid phosphatase is still present in purified preparations of the enzyme. Therefore this effect cannot be explained by an effect of calcium on a subcellular structure associated with acid phosphatase activity.

About postmortem material and enzyme studies: Even under the best possible conditions, we feel that postmortem material, as obtained in most hospitals, is unsatisfactory, especially from patients with chronic diseases. Accidental deaths may provide more suitable material, but in each case, experimental study will have to prove the validity of conclusions drawn when this type of material is used. The most satisfactory material is fresh surgical specimens, obtained by collaboration with the surgeon. One of the very real difficulties slowing down enzyme studies on human tumors is that of regular procurement of suitable material. This is why much interest is centering now on the possibility of establishing human tumor tissue "banks" in which representative samples of human tumor, preserved under optimal conditions, could be made available to biochemists and clinical investigators. I believe that the establishment of such "banks" would serve a very important function.

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Chairman's Closing Remarks

Dr. Jensen: The time has come to bring this conference to a close. We are grateful to Dr. Scott and his committee for organizing the program, and to the staff members from the National Cancer Institute for arranging the conference and all the details that go with it. I would like to thank all of you for coming to participate in the workshop and for helping to make it a success. It has been a great pleasure to have had you with us.

GLOSSARY

Note: The glossary is for the convenience of the reader who encounters unfamiliar terms in this book and wants at least a helpful synonym. Terms which appear self-explanatory, which are well defined in context, or which have no common synonym are not included. Synonyms for steroids are given principally in the IUPAC System to show interrelationships.

A

Term	*Synonym or definition*
17-Acetoxyprogesterone	17-Hydroxypregn-4-ene-3,20-dione, acetate
Acetylcholine	Acetylcholine chloride
N-Acetyl neuraminic acid	Sialic acid
2-Acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene	7-Acetyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2-(3H)-phenanthrone Ro-2-7239
ACTH	Adrenocorticotropic hormone Corticotropin
17α-Allyl-19-nortestosterone	17α-Allyl-17-hydroxyestr-4-en-3-one
Aminopyrine	4-Dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one
AMP	Adenosine monophosphate
$\Delta^{1,4}$-Androstadiene-3,17-dione	Androsta-1,4-diene-3,17-dione
$\Delta^{1,4}$-Androstadien-17-ol-3-one	17β-Hydroxyandrosta-1,4-dien-3-one
Androstanediol	5α-Androstane-3α,17β-diol
Androstanedione	5α-Androstane-3,17-dione
Androstan-17β-ol-3-one	17β-Hydroxy-5α-androstan-3-one Dihydrotestosterone
Δ^1-Androstene-3,17-diol	5α-Androst-1-ene-3,17-diol
Androstenediol	Androst-5-ene-3β,17β-diol
Δ^1-Androstene-3,17-dione	5α-Androst-1-ene-3,17-dione
Δ^4-Androstene-3,17-dione	Androst-4-ene-3,17-dione
Δ^4-Androstene-3,17-dione-7-H^3	Androst-4-ene-3,17-dione, 7-H^3
Δ^1-Androsten-17-ol-3-one	17β-Hydroxy-5α-androst-1-en-3-one
Androsterone	3α-Hydroxy-5α-androstan-17-one
ATHF	3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one Allo-tetrahydrocortisol
ATP	Adenosinetriphosphate 5′-Adenyldiphosphoric acid

B

Benzpyrene	3,4-Benzpyrene or 1,2-Benzpyrene
17α-Butyl-19-nortestosterone	17α-Butyl-17-hydroxyestr-4-en-3-one

C

Carbestrol	3-Ethyl-4-(*p*-methoxyphenyl)-2-methyl-3-cyclohexene-1-carboxylic acid
Ceruloplasmin	A copper-protein
6α-Chloro-17-acetoxyprogesterone	6α-Chloro-17-hydroxypregn-4-ene-3,20-dione, acetate
6-Chloro-Δ^6-dehydro-17-hydroxyprogesterone	6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione

Term	*Synonym or definition*
Chlormadinone	6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione, acetate
9α-Chloro-17α-methyltestosterone	9α-Chloro-17β-hydroxy-17-methylandrost-4-en-3-one
Cholesterol	Cholest-5-en-3β-ol
Choline	Trimethyl-β-hydroxyethylammonium hydroxide
Cinophen	An alkaloid from Cinchona bark
Civetolone	17-Hydroxycivetone
Civetone	Cycloheptadec-9-en-1-one
Clomiphene	1-[*p*-(β-diethylaminoethoxy)phenyl]-1,2-diphenyl-2-chloroethylene Chloramiphene MRL-41
Co A	Coenzyme A, a co-factor in enzymatic acetyl transfer reactions
Cortisol	11β,17,21-Trihydroxypregn-4-ene-3,20-dione Hydrocortisone Compound F
Cortisone	17,21-Dihydroxypregn-4-ene-3,11,20-trione Compound E
CTP	Cytidine triphosphate
CTP ^{32}	CTP (cytidine triphosphate) labeled in the innermost (ester) phosphate with P^{32}
	D
Decadron	*See* Dexamethasone
6-Dehydroestrone	3-Hydroxyestra-1,3,5(10),6-tetraen-17-one
Dehydroisoandrosterone	3β-Hydroxyandrost-5-en-17-one Dehydroepiandrosterone
1-Dehydrotestololactone	17a-Oxa-D-homoandrosta-1,4-diene-3,17-dione Δ^1-Testololactone
Delalutin	17-Hydroxypregn-4-ene-3,20-dione, hexanoate [= –, caproate] 17-Hydroxyprogesterone, hexanoate [= –, caproate]
Deoxycorticosterone	21-Hydroxypregn-4-ene-3,20-dione DOC
Dexamethasone	9α-Fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione 9α-Fluoro-16α-methylprednisolone Decadron Hexadecadrol
Dianabol	17β-Hydroxy-17-methylandrosta-1,4-dien-3-one 1-Dehydro-17-methyltestosterone Methandrostenolone
Diethylstilbestrol	α,α′-Diethyl-4,4′-stilbenediol Stilbestrol
Diethylstilbestrol diphosphate	α,α′-Diethyl-4,4′-stilbenediol diphosphate, disodium salt Stilphostrol
Dihydroisosteviol	*See* Steviol
Dihydrotestosterone	17β-Hydroxy-5α-androstan-3-one Androstan-17β-ol-3-one
Dimethylstilbestrol	α,α′-Dimethyl-4,4′-stilbenediol
Dithizone	Diphenylthiocarbazone

Term	*Synonym or definition*
DNA	Deoxyribonucleic acid
Dromostanolone	17β-Hydroxy-2α-methyl-5α-androstan-3-one 2α-Methyldihydrotestosterone Adroyd
	E
16,17-Epoxy-21-acetoxy-Δ^4-pregnene-3,20-dione	16α,17-Epoxy-21-hydroxypregn-4-ene-3,20-dione, acetate 11-Deoxy-16α,17-epoxycorticosterone, acetate
16,17-Epoxy-3β-acetoxy-Δ^5-pregnen-20-one	16α,17-Epoxy-3β-hydroxypregn-5-en-20-one, acetate
16,17-Epoxyprogesterone	16α,17-Epoxypregn-4-ene-3,20-dione
Equilin	3-Hydroxyestra-1,3,5(10),7-tetraen-17-one
Ergothioneine	Betaine of thiolhistidine
Estinyl	*See* Ethynyl estradiol
Estradiol	Estra-1,3,5(10)-triene-3,17β-diol Estradiol-17β
"α-Estradiol"	Estradiol-17β
Estradiol-17α	Estra-1,3,5(10)-triene-3,17α-diol
16β-Estradiol	Estra-1,3,5(10)-triene-3,16β-diol
6,7-Tritiated estradiol	Estra-1,3,5(10)-triene-3,17β-diol, 6,7-H^3
17-Tritiated estradiol	Estra-1,3,5(10)-triene-3,17β-diol, 17-OH^3
Estradiol-17β, 3-benzoate	Estra-1,3,5(10)-triene-3,17β-diol, 3-benzoate
Estradiol-17β, 17-chloroethynyl-	17α-Chloroethynylestra-1,3,5(10)-triene-3,17-diol, 21-Chloro-19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol
Estradiol-17β, 6-dehydro-	Estra-1,3,5(10),6-tetraene-3,17β-diol
Estradiol-17β, 6α-hydroxy-, triacetate	Estra-1,3,5(10)-triene-3,6α,17β-triol, triacetate
Estradiol-17β, 6β-hydroxy-, triacetate	Estra-1,3,5(10)-triene-3,6β,17β-triol, triacetate
Estradiol-17β, 11β-hydroxy-	Estra-1,3,5(10)-triene-3,11β,17β triol
$\Delta^{5(10)}$-Estren-17-ol-3-one	17β-Hydroxyestr-5(10)-en-3-one
Estriol	Estra-1,3,5(10)-triene-3,16α,17β-triol
16β-Estriol, 3-methyl ether	3-Methoxyestra-1,3,5(10)-triene-16β,17β-diol
Estrololactone	3-Hydroxy-17a-oxa-D-homoestra-1,3,5(10)-trien-17-one 3,13α-Dihydroxy-13,17-secoestra-1,3,5(10)-trien-17-oic acid, δ-lactone
Estrone	3-Hydroxyestra-1,3,5(10)-trien-17-one Ketohydroxyestrin
Estrone, 6-dehydro-	*See* 6-Dehydroestrone
Estrone, K sulfate	3β-Hydroxyestra-1,3,5(10)-trien-17-one, potassium sulfate
17α-Ethylandrostan-17-ol-3-one	17α-Ethyl-17-hydroxy-5α-androstan-3-one 17β-Hydroxy-5α,17-pregnan-3-one
17α-Ethyl-Δ^1-androsten-17-ol-3-one	17α-Ethyl-17-hydroxy-5α-androst-1-en-3-one 1-Dehydro-4,5α-dihydro-17-ethyltestosterone
17α-Ethyl-Δ^4-androsten-17-ol-3-one	17α-Ethyl-17-hydroxyandrost-4-en-3-one Ethyltestosterone
17α-Ethyl-19-norandrostan-17-ol-3-one	17α-Ethyl-17-hydroxy-5α-estran-3-one
17α-Ethyl-19-nor-Δ^4-androsten-17-ol-3-one	17α-Ethyl-17-hydroxyestr-4-en-3-one 17-Ethyl-19-nortestosterone
Ethyltestosterone	17α-Ethyl-17-hydroxyandrost-4-en-3-one

Term	*Synonym or definition*
Ethynyl estradiol	17α-Ethynylestra-1,3,5(10)-triene-3,17-diol 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol Estinyl
17-Ethynyl-19-nortestosterone	17α-Ethynyl-17-hydroxyestr-4-en-3-one Norlutin Norethisterone Norethindrone
17α-Ethynyltestosterone	17α-Ethynyl-17-hydroxyandrost-4-en-3-one 17β-Hydroxy-17-pregn-4-en-20-yn-3-one
Etiocholanolone	3α-Hydroxy-5β-androstan-17-one 3α-Hydroxyetiocholan-17-one
	F
9α-Fluorocortisol	9-Fluoro-11β,17,21-trihydroxypregn-4-ene-3,20-dione 9-Fluoro-17-hydroxycorticosterone
2α-Fluorodihydrotestosterone benzoate	2α-Fluoro-17β-hydroxy-5α-androstan-3-one, benzoate
21-Fluoro-11-hydroxyprogesterone	21-Fluoro-11β-hydroxypregn-4-ene-3,20-dione
2-Fluoro-17-methyltestosterone	2α-Fluoro-17β-hydroxy-17-methylandrost-4-en-3-one
6α-Fluoroprednisolone	6α-Fluoro-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione
21-Fluoro-Δ^4-pregnene-3,20-dione	*See* 21-Fluoroprogesterone
21-Fluoroprogesterone	21-Fluoropregn-4-ene-3,20-dione
2-Fluorotestosterone	2α-Fluoro-17β-hydroxyandrost-4-en-3-one
Fluoxymesterone	*See* Halotestin
Folliculin	Estrone
Formazan	Monoformazan, a reduction product of triphenyltetrazolium, of deep-red color
FSH	Follicle-stimulating hormone
	G
GH	Growth hormone
Glycyrylphosphorylcholine	Glycyryl ester of (2-hydroxyethyl)-trimethylammonium chloride phosphate
Gonadotropin	Human chorionic gonadotropin HCG Antuitrin S
GTP	Guanosinetriphosphate
	H
Halotestin	9α-Fluoro-11β,17β-dihydroxy-17-methylandrost-4-en-3-one 9α-Fluoro-11β-hydroxy-17-methyltestosterone Fluoxymesterone
HCG	Human chorionic gonadotropin
Hemocuprein	A copper-protein
Hexadecadrol	*See* Dexamethasone
Hexestrol	4,4′-(1,2-diethylethylene)-diphenol
Hexestrol, 2-fluoro-	2-Fluoro-4,4′-(1,2-diethylethylene)-diphenol
Hydrocortisone	11β,17,21-Trihydroxypregn-4-ene-3,20-dione Cortisol

Term	*Synonym or definition*
3α-Hydroxyandrostan-17-one	3α-Hydroxy-5α-androstan-17-one Androsterone
6β-Hydroxyandrostenedione	6β-Hydroxyandrost-4-ene-3,17-dione
11β-Hydroxy-21-fluoroprogesterone	21-Fluoro-11β-hydroxypregn-4-ene-3,20-dione
11α-Hydroxyprogesterone	11α-Hydroxypregn-4-ene-3,20-dione
11β-Hydroxyprogesterone	11β-Hydroxypregn-4-ene-3,20-dione
16-Hydroxyprogesterone	16α-Hydroxypregn-4-ene-3,20-dione
17α-Hydroxyprogesterone	17-Hydroxypregn-4-ene-3,20-dione
17α-Hydroxyprogesterone hexanoate	17-Hydroxypregn-4-ene-3,20-dione, hexanoate [= –, caproate] Delalutin
6β-Hydroxytestosterone	6β,17β-Dihydroxyandrost-4-en-3-one
5-Hydroxytryptamine	Serotonin
	I
ICSH	Interstitial cell-stimulating hormone
	K
11-Keto-Δ^{16}-dehydroprogesterone	Pregna-4,16-diene-3,11,20-trione
Ketohydroxyestrin	Estrone
11-Keto-17α-hydroxyprogesterone	17-Hydroxypregn-4-ene-3,11,20-trione
	L
Lecithin	A mixture of the diglycerides of stearic, palmitic, and oleic acids linked to phosphoryl choline
LH	Luteinizing hormone
LTH	Luteotropic hormone Prolactin
	M
Malononitrile mustard	(*p*-[bis(2-Chloroethyl)amino]-benzylidene)-malononitrile
6-MAP	*See* Provera
Medrol	*See* 6α-Methylprednisolone
Medroxyprogesterone	*See* Provera
MER-25	1-[*p*-(2-Diethylaminoethoxy)-phenyl]-2-(*p*-methoxyphenyl)-1-phenylethanol
17α-(1-Methallyl)-19-nortestosterone	17β-Hydroxy-17-(1-methylallyl)-estr-4-en-3-one 17α-(1-Methylallyl)-19-norandrost-4-en-3-one
17α-(2-Methallyl)-19-nortestosterone	17β-Hydroxy-17-(2-methylallyl)-estr-4-en-3-one 17α-(2-Methylallyl)-19-norandrost-4-en-3-one
Methandrostenolone	*See* Dianabol
6α-Methyl-17α-acetoxyprogesterone	17-Hydroxy-6α-methylpregn-4-ene-3,20-dione, acetate Provera 6-MAP Medroxyprogesterone
17α-Methyl-$\Delta^{1,4}$-androstadien-17-ol-3-one	*See* Dianabol
17α-Methylandrostan-17-ol-3-one	17β-Hydroxy-17-methyl-5α-androstan-3-one
17α-Methyl-Δ^{1}-androsten-17-ol-3-one	17β-Hydroxy-17-methyl-5α-androst-1-en-3-one
17α-Methyl-Δ^{4}-androsten-17-ol-3-one	17β-Hydroxy-17-methylandrost-4-en-3-one

Term	*Synonym or definition*
Methylcholanthrene	20-Methylcholanthrene or 3-Methylcholanthrene
2α-Methyldihydrotestosterone	*See* Dromostanolone
2α-Methyldihydrotestosterone propionate	17β-Hydroxy-2α-methyl-5α-androstan-3-one, propionate Dromostanolone propionate
17α-Methylestradiol	17α-Methylestra-1,3,5(10)-triene-3,17-diol
2α-Methyl-17β-hydroxy-19-nor-Δ^{4}-androsten-3-one	17β-Hydroxy-2α-methylestr-4-en-3-one 2α-Methyl-19-nortestosterone
2α-Methyl-11-ketoprogesterone	2α-Methylpregn-4-ene-3,11,20-trione
6α-Methyl-11-ketoprogesterone	6α-Methylpregn-4-ene-3,11,20-trione
2α-Methyl-19-nortestosterone	17β-Hydroxy-2α-methylestr-4-en-3-one
17α-Methyl-19-nortestosterone	17β-Hydroxy-17-methylestr-4-en-3-one
6α-Methylprednisolone	11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione Medrol
Methyltestosterone	17β-Hydroxy-17-methylandrost-4-en-3-one
MRL-41	*See* Clomiphene
	N
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
Nitrogen mustard	2,2′-Dichloro-*N*-methyldiethylamine, hydrochloride
Norethindrone	*See* 17-Ethynyl-19-nortestosterone
Norethisterone	*See* 17-Ethynyl-19-nortestosterone
Norlutin	*See* 17-Ethynyl-19-nortestosterone
A-Norprogesterone	A-Norpregn-3(5)-en-2,20-dione
19-Norprogesterone	19-Norpregn-4-ene-3,20-dione
19-Nortestosterone	17β-Hydroxyestr-4-en-3-one 17β-Hydroxy-19-norandrost-4-en-3-one
	P
PAS	Periodic acid-Schiff's reagent
pCpCpA	"Terminal cytidylate–cytidylate–adenosine sequence in soluble RNA"
Phosphatidal choline	*See* Plasmalogen
Phosphorylcholine	(2-Hydroxyethyl)-trimethylammonium chloride phosphate
Plasmalogen	An acetal phosphatide of choline
Poly U	Polyuridylic acid
Poly UC	"Randomly ordered copolymer of uridylic and cytidylic acids"
Poly UG	"Randomly ordered copolymer of uridylic and guanylic acids"
$\Delta^{4,16}$-Pregnadiene-3,11,20-trione	Pregna-4,16-diene-3,11,20-trione
$\Delta^{4,17(20)}$-*cis*-Pregnadien-21-oic acid, 3,11-diketo-, methyl ester	3,11-Dioxopregna-4,17(20)-*cis*-dien-21-oic acid, methyl ester
Pregnenolone	3β-Hydroxpregn-5-en-20-one
Progesterone	Pregn-4-ene-3,20-dione
Progesterone-7-H^3	Pregn-4-ene-3,20-dione, 7-H^3

Term	*Synonym or definition*
Progesterone, 11β,17-dihydroxy-6α-methyl-	11β,17-Dihydroxy-6-α-methylpregn-4-ene-3,20-dione
Progesterone, 21-fluoro-11β,17-dihydroxy-6α-methyl-	21-Fluoro-11β,17-dihydroxy-6α-methylpregn-4-ene-3,20-dione
Progesterone, 17α-hydroxy, hexanoate	*See* Delalutin
Progesterone, 17-hydroxy-6-methylene-, acetate	17-Hydroxy-6-methylenepregn-4-ene-3,20-dione, acetate
Prolactin	Luteotropic hormone LTH
17α-Propyl-19-nortestosterone	17β-Hydroxy-17-propylestr-4-en-3-one
Prostaglandin E	2-(6-Carboxyhexyl)-3-(3-hydroxyocten-1-yl)-4-hydroxycyclopentanone
Prostaglandin F	Reduction product of Prostaglandin E
Provera	17-Hydroxy-6α-methylpregn-4-ene-3,20-dione, acetate 6-MAP 6α-Methyl-17α-acetoxyprogesterone Medroxyprogesterone
	R
RNA	Ribonucleic acid
m-RNA	Messenger RNA
ms-RNA	Microsomal RNA
n-RNA	Nuclear RNA
s-RNA	Soluble RNA
Ro-2-7239	2-Acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydrophenanthrene 7-Acetyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrone
	S
Serotonin	5-Hydroxytryptamine 3-(2-Aminoethyl)-5-indolol
Sialic acid	*N*-Acetylneuraminic acid
Spermidine	Aminopropyltetramethylene diamine
Spermine	Diaminopropyltetramethylene diamine
Steviol	An aglucone from stevioside on hydrolysis *See* Stevioside
Stevioside	A glucoside component in Rebaudin, extract from leaves of *Stevia rebaudiana*
STH	Somatotropic hormone Somatotropin
Stilbestrol	α,α′-Diethyl-4,4′-stilbenediol Diethylstilbestrol
Stilphostrol	α,α′-Diethyl-4,4′-stilbenediol diphosphate, disodium salt Diethylstilbestrol diphosphate
	T
TACE	Chlorotris(*p*-methoxyphenyl)-ethylene Chlorotrianisene
TEM	2,4,6-Tris(1-aziridinyl)-*s*-triazine Triethylene melamine

Term	*Synonym or definition*
Δ^1-Testololactone	17a-Oxa-D-homoandrosta-1,4-diene-3,17-dione 1-Dehydrotestololactone
Testosterone	17β-Hydroxyandrost-4-en-3-one Δ^4-Androsten-17-ol-3-one
4-C^{14}-Testosterone	17β-Hydroxyandrost-4-en-3-one, 4-C^{14}
1,2-Tritiated testosterone	17β-Hydroxyandrost-4-en-3-one, 1,2-H^3
Testosterone, 9-chloro-11-oxo-17-methyl-	9α-Chloro-17β-hydroxy-17-methylandrost-4-ene-3,11-dione
Testosterone, 4,5α-dihydro-2α-methyl-	*See* Dromostanolone
Testosterone enanthate	17β-Hydroxyandrost-4-en-3-one, heptanoate Testosterone heptanoate
Testosterone, 6-methylene-, acetate	17β-Hydroxy-6-methyleneandrost-4-en-3-one, acetate
Testosterone propionate	17β-Hydroxyandrost-4-en-3-one, propionate
THE	3α,17,21-Trihydroxy-5β-pregnane-11,20-dione Tetrahydrocortisone
THF	3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one Tetrahydrocortisol
Thiolhistidine betaine	L(+) Ergothioneine Ergothioneine
Thyrotropin	Thyrotropic hormone TSH
Thyroxine	3,5,3′,5′-Tetraiodothyronine
TPN	Triphosphopyridine nucleotide
Triphenyltetrazolium	2,3,5-Triphenyl-2*H*-tetrazolium chloride Triphenyltetrazolium chloride
TSH	Thyrotropic hormone Thyrotropin

U

UTP	Uridinetriphosphate

V

Versene	Ethylenediamine tetraacetic acid, tetrasodium salt
17α-Vinyl-19-nortestosterone	17β-Hydroxy-17-vinylestr-4-en-3-one 17β-Hydroxy-19-nor-17-pregna-4,20-dien-3-one

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